

Priorities in astronomy

US astronomers are being forced to revisit their widely admired system for ranking large projects.

In the beginning, astronomers believe, our tiny Universe underwent ‘inflation’ — a period of growth so rapid that it separated sections of the cosmos from each other forever.

Something similar has happened in astronomy itself over the past half-century. In the 1950s, the field was a primordial ball of scientists using a handful of modest telescopes to peer at stars and galaxies. Today, thousands of them use telescopes, radio arrays, balloons and satellites to learn about black holes, extrasolar planets, star nurseries or the early Universe.

In the United States, a process known as the decadal review has guided this rapid expansion. The review is compiled by a committee, gathered under the auspices of the National Academy of Sciences, and lists which instruments should be built over the next decade, along with estimates of their cost. It has served US astronomy well, and other capital-intensive disciplines of science have sought to learn from its example. But the review process is under increasing strain (see page 386). Most projects in the 2001 review have been severely delayed, and some have been “indefinitely deferred”. As the next decadal review gets under way, astronomers are wondering how to salvage its tattered credibility.

The problem arises, first and foremost, because astronomy funding’s own period of inflation is drawing to a close. Budget constraints at federal science agencies, most notably NASA, have seen to that. The slowdown is compounded by the steadily growing size, and therefore cost, of the decadal review’s project wish-list.

And as happened in the early Universe, the rapid early expansion of astronomy has left its component parts cut off from one another. Astronomers have organized themselves by the type of starlight they study — X-rays are one subdiscipline, infrared another. The partition made sense because each subfield requires different types of telescopes. But it has added a sectarian element to the review process, as various subpanels, organized by light type, have fought to

secure the place of their field’s instrument in the review document.

The need to placate these different groups has forced the review’s outcome to become unwieldy. In 1972, for example, the review simply listed eleven favoured projects in rank order. By 2001, it was placing projects in subcategories of large, medium and small, and whether they were space- or ground-based, before ranking them. This obtuse system identified no less than five different projects as astronomy’s ‘top priority’.

Astronomers are therefore looking at ways to restore some of the simplicity that was the review’s great strength. One approach may be to turn to the science. Rather than dividing astronomy by wavelength, reviewers could decide which areas of investigation are likely to prove most promising — the study of ‘dark energy’, for example. Researchers from each subfield could then come together on subpanels to establish which instruments could best address the problem, and how each subfield could best contribute.

The basic idea is not without precedent: US Earth scientists are already using it in their own, inaugural decadal review. Their National Academies panel is organized by categories such as weather, climate, hydrology, natural hazards and solid Earth research, with the weather panel, for example, incorporating hydrologists, atmospheric physicists and oceanographers. It expects to produce a prioritized list of instruments, some of which will be shared by subdisciplines.

Such a change in outlook is not a panacea for astronomy. Given the budget constraints, the next review will have to make tough choices and exclude some excellent projects. But reorganizing the decadal review along scientific lines would go some way towards ensuring its continued relevance in astronomy’s post-inflationary epoch. ■

“Astronomers are looking for ways to restore some of the simplicity that was the review’s great strength.”

A German academy

In seeking to build a credible national academy from scratch, Germany faces a tough challenge.

For historical reasons, modern Germany has no national scientific academy along the lines of the Royal Society in London or the US National Academies. It is now seeking to design one — but building a reputable academy from scratch may be more difficult than it at first appears.

Clearly, the academy’s value will ultimately be measured by the quality of the reports and recommendations it produces, and by the influence it manages to yield. In a pluralistic society, no single organization can claim to be a central committee of truth. But the

functioning of modern nations correlates closely with their handling of technical, medical and scientific problems. They need to receive well-considered scientific advice that has a real chance of making a difference in the political arena.

The fact that Germany is building an academy from scratch gives it the opportunity to learn from others, and to do it right. Scientific academies have sometimes carried the whiff of exclusive clubs where scientists who are usually elderly, white and male retire to indulge in arcane intellectual pleasures. It is a tough balancing act to ensure that an academy will respect experience and acquired wisdom, while also reflecting the diversity — in age, gender, background and outlook — of today’s scientific community.

Germany’s existing learned societies include seven regional academies, which are most active in the humanities and social sciences, and the 350-year-old Leopoldina, which represents the medical and



natural sciences in the German-language region, including Austria and Switzerland. The East German Academy of Sciences, which until 1990 was the main scientific institution in the communist German Democratic Republic, has been closed down.

None of these organizations can claim to speak for German science as a whole, either at home or abroad. Nor can they fulfil the advisory role undertaken by national academies elsewhere. The Deutsche Forschungsgemeinschaft (DFG), Germany's main research agency, has done some of this in the past. But as a funding agency affiliated to the government, it cannot act as the independent voice of the scientific community in Germany.

A national academy has been on the radar of Germany's scientific leaders ever since reunification. In 2004 the Wissenschaftsrat — a council of the great and the good that currently advises the government on science policy — asked the regional academies to come up with their ideas for one. Under the academies' as-yet-unpublished proposal, the national academy would comprise a 200-strong council, appointed by the regional academies. It would generate advice for the government through ad-hoc working groups with appropriate

external expertise. The details of how this will be done are still being debated within the scientific community, but the outline proposal will be considered by a meeting of the German states' science ministers next month.

Operating the academy will require modest government funding. It will need enough money to establish a robust technical staff, to help compile reports and organize public outreach. The manner in which it is funded will need to be designed to ensure that the academy retains political independence. One route would be for the academy to draw its money from the budget of the (politically neutral) federal president's office. The architects of the plan have yet to iron out all of these details.

Ultimately, a successful academy will have to earn not just the backing of scientists, but the trust of large segments of the public. It will be essential for the new body to proactively seek the engagement of the public from the outset. By giving due consideration to contentious issues, the academy should benefit German society by encouraging a healthy level of scientific discourse and improved public understanding of science. ■

Reforms on drug safety

Critics of the US Food and Drug Administration have a valid point.

The US drug safety system is outdated, weak, disorganized and seriously underfunded, according to no less an authority than the Institute of Medicine (IOM). The Food and Drug Administration (FDA) has neither the money nor the muscle to police the safety of drugs already on the market, says a report released by the institute last week.

The FDA itself asked the IOM, which represents the most eminent figures in US medical research, to look into its drug-regulation process in 2004 after the withdrawal of the painkiller Vioxx, which was found to increase the risk of heart attacks and strokes (see *Nature* 432, 537; 2004). Vioxx provided an example of what can happen when drugs originally tested in limited numbers of people in clinical trials are put to work in the real world.

Subsequent examination has confirmed that the FDA's post-market safety efforts are paltry, particularly compared with its exhaustive pre-approval drug-evaluation process. That impression is confirmed by the IOM report, which makes more than two dozen recommendations for strengthening the US drug safety system.

The IOM committee, chaired by Sheila Burke, chief operating officer at the Smithsonian Institution, notes that the current balance of priorities at the FDA reflects an earlier era, when prescription medicines were usually taken one at a time, for short periods. It is less appropriate for today's chronically medicated, rapidly ageing, multiple-pill-popping population.

Many of the IOM's recommendations focus on strengthening the FDA's approach to ensuring the safety of drugs already on the market. The committee wants Congress to give the FDA the authority to fine companies that fail to run promised studies of their drugs once they

are for sale. Companies commit to carrying out such studies when a drug is approved, but all too often these promises go unfulfilled.

The IOM also asks Congress to grant the FDA power to compel companies to change drug labels when new safety concerns arise. Under current law, the FDA can request but not demand these changes, often leading to protracted negotiations with drug firms. In the case of Vioxx, it spent 14 months getting a reference to heart-attack risks added to the label, years before the drug was pulled.

The report says that newly approved drugs should carry a warning label — perhaps a black triangle — for their first two years or so on the market, to warn consumers about the limited state of knowledge on the drug. Direct advertising to consumers should also be restricted in this early period, it argues.

The IOM also proposes that a fixed, six-year term should be established for the post of FDA commissioner, in an attempt to detach it from the vicissitudes of the four-year presidential election cycle, and hence from party politics.

Then there is the matter of money. The IOM wants Congress to give the FDA far more resources to monitor the safety of existing drugs. Rather than this being financed by the drug industry through user fees, it is emphatic that the money should come from the public purse, to make sure that the regulator is seen to be independent of excessive industry influence.

The radical changes that the IOM is recommending may face an uphill struggle in Congress. But public concerns about drug safety need to be addressed, and senior figures in both parties, from Senator Chuck Grassley (Republican, Iowa) to Senator Edward Kennedy (Democrat, Massachusetts), have the issue firmly in their sights. Congress should take some of the bold steps that the IOM report is suggesting, in order to restore the FDA's reputation for firm but fair drug regulation. ■

"Public concerns about drug safety need to be addressed, and senior figures in both parties have the issue in their sights."

RESEARCH HIGHLIGHTS

Male eggs laid last

Proc. Natl Acad. Sci. USA **103**, 14406–14411 (2006)

House finches (*Carpodacus mexicanus*, pictured) have a smart strategy to spare their chicks from parasites. Females whose nests are infested with mites lay female-bearing eggs first and male ones last. Male chicks then grow faster than the females.

The strategy helps to ensure that reproductive output is damaged as little as possible by the infestation, say researchers led by Alexander Badyaev of the University of Arizona in Tucson. Male chicks are more vulnerable to being killed by mites, so laying them last and giving them more of a growth spurt minimizes their time in the nest while allowing them to grow to full fledging size in a shorter time.



A. BADYAEV

CANCER BIOLOGY

Breaking and entering

Genes Dev. doi:10.1101/gad.1451806 (2006)

To spread, or metastasize, around the body, cancer cells must escape from their site of origin. Doing so requires crossing the basement membrane, a specialized barrier of connective tissue.

Stephen Weiss and his colleagues at the University of Michigan, Ann Arbor, have identified three enzymes that allow tumours to degrade proteins in the basement membrane, opening a hole through which cancer cells can escape. Expression of any one of the three enzymes is enough to chew through the barrier.

Once the hole is there, tumour cells squeeze through, amoeba-style. Knowing the enzymes that puncture the basement membrane could enable future cancer therapies to target this process.

PHYSICS

Silicon success story

Phys. Rev. Lett. **97**, 116101 (2006)

Researchers at Vanderbilt University in Nashville, Tennessee, have unpicked one of the reasons why silicon has been so successful in electronics.

When silicon is heated to temperatures above 800 °C, its surface layer becomes oxidized, turning into silicon dioxide. The interface between the silicon and the insulating oxide layer is exceptionally smooth, which is essential to making high-performance electrical devices.

Leonidas Tsetseris and Sokrates Pantelides modelled the oxidation process. They found that the random deposition of oxygen, which

might be expected to give a rough interface, is counteracted by relaxation processes (such as diffusion of the oxygen) activated by the high temperatures.

ENVIRONMENTAL SCIENCE

Storm surge

Science doi:10.1126/science.1129116 (2006)

Hurricanes seem to be the main factor in replenishing inorganic sediments along the US Gulf Coast. This finding will have to be taken into account in plans to restore the region's wetlands by diverting rivers into the marshes — an effort meant, in part, to increase sedimentation rates.

The results come from a team led by R. Eugene Turner, of Louisiana State University in Baton Rouge, which walked, boated and helicoptered along the ravaged shoreline after Hurricanes Katrina and Rita hit Louisiana and Texas last autumn. Mud samples revealed the huge amounts of inorganic sediments left by the storms — 227 times the amount introduced by one river

diversion built for wetland restoration.

The health of marshes depends on many factors, however, and river diversions may bring other benefits.

IMMUNOLOGY

To the root of the problem

Cell **126**, 1121–1133 (2006)

A molecule called ROR γ t helps to promote inflammation and might be targeted by new drugs to fight autoimmune diseases such as multiple sclerosis and rheumatoid arthritis.

A certain population of immune cells, called Th17 cells, is known to promote inflammation and be a key driver of autoimmune disease. ROR γ t directs the development of these cells, say Daniel Cua at Schering-Plough BioPharma in Palo Alto, California, Dan Littman at the Skirball Institute of Biomolecular Medicine in New York and their colleagues. They found that mice lacking ROR γ t are resistant to autoimmune disease and have fewer Th17 cells.

ROR γ t triggers the production of inflammation-provoking cytokines and may normally help to control the numerous bugs in the gut.

NEUROSCIENCE

Insight into Alzheimer's

Science **10.1126/science.1132341** (2006);

Science **313**, 1781–1784 (2006)

Two studies give new insight into the biology of β -amyloid, the protein that forms clumps in the brains of Alzheimer's patients.

One team studied an enzyme called β -secretase, which is targeted by current drug therapies. It is needed for β -amyloid to form, but its normal function was unknown.



SCIENCE

The team found that it is essential for the formation of myelin, a fatty insulating substance, around peripheral nerves.

In a separate study, researchers suggest that there might be different 'strains' of β -amyloid with different biological properties. They injected diseased brain extracts into unaffected mouse brains, causing β -amyloid clumps to form. The pathology that develops depends on both the source of the agent and the host into which it is injected.

GEOSCIENCE

Modelling Earth's interior

Geophys. Res. Lett. **33**, L18301 (2006)

Satellites have been used to probe the electrical conductivity of Earth's interior. Researchers analysed data from three geomagnetism satellites to model how Earth's conductivity varies with depth — information that can help constrain the properties of the mantle.

The model resembles those created using data gathered by ground-based observatories, report Alexei Kuvshinov and Nils Olsen of the Danish National Space Center in Copenhagen. Their result is less noisy than previous models based on satellite data because they used data collected over a longer time period, totalling five years.

The same could be done for other planets, the researchers point out, providing a new tool for planetary exploration.

CHEMISTRY

Speed search

Chem. Comm. doi:10.1039/b609138e (2006)

Chemists have borrowed the idea of 'cultural evolution' to speed up the algorithms that determine crystal structures from X-ray diffraction patterns.

Current evolutionary algorithms start with trial crystal structures, then randomly mutate properties such as the position of the atoms. The structures that best match the X-ray patterns are then mutated and 'mated' to optimize the fit with the data. Such algorithms therefore follow the principles of genetic evolution.

Cultural evolution introduces the idea that past behaviour constricts that of future generations, a phenomenon seen in fashion. Maryjane Tremayne and Samantha Chong from the University of Birmingham, UK, applied this concept to crystal-structure searches. They showed that restricting the limits between which the crystal parameters vary after each trial generation doubles the speed with which the genetic algorithm reaches its solution.

MATERIALS SCIENCE

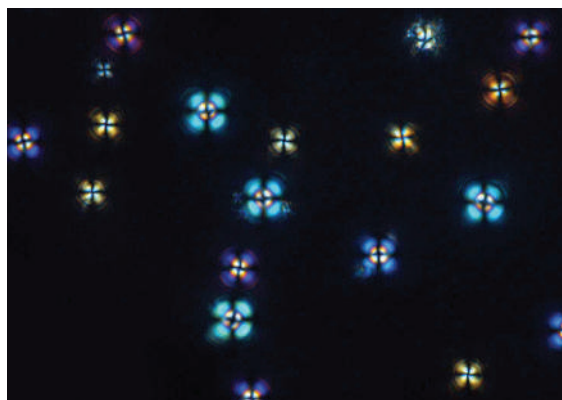
Liquid-crystal sensors

Nano Lett. doi:10.1021/nl061604p (2006)

Liquid-crystal droplets wrapped in polymers could provide a tool to track changes in biological systems, researchers suggest.

Nick Abbott of the University of Wisconsin, Madison, Frank Caruso of the University of Melbourne, Australia, and their colleagues showed that the polymer coat slows the liquid crystals' response to a chemical that changes the orientation of its molecules, suggesting that the coats can be used to influence such interactions. The orientation change is easily tracked because it alters how the droplets look under polarized light (pictured below).

Abbott and Caruso suggest that the interaction of biological molecules with the droplets' coating, which could be engineered to contain enzyme substrates, may also change the structure of the liquid-crystal core, turning the droplets into sensors.



BIOTECHNOLOGY

Switch for yeast's cell cycle

Angew. Chem. Int. Edn **45**, 6322–6325 (2006)

A light-controlled switch that operates on the cell cycle of budding yeast, *Saccharomyces cerevisiae*, will pave the way for novel experiments, report researchers from the University of Chicago, Illinois.

Conventionally, researchers synchronize *S. cerevisiae* for cell-cycle studies by adding the pheromone α -factor, which stalls the yeast cell on the brink of the division process. To restart the cycle, they wash the pheromone away.

Stephen Kron and his colleagues redesigned α -factor to eliminate the imprecise washing step. They swapped one amino acid in the pheromone for a chemical group that can be cleaved by ultraviolet light. Adding this α -factor analogue to *S. cerevisiae* arrested the yeast's cell cycle; brief exposure to ultraviolet light restarted it.

AM. CHEM. SOC.

JOURNAL CLUB

Sossina Haile
California Institute of
Technology, Pasadena

A materials scientist argues for an alternative to hydrogen fuel.

Much has been said about the 'hydrogen economy' as a way to a clean, sustainable and geopolitically sound energy future. But hydrogen is not an energy source, merely an energy carrier. The fact that hydrogen does not produce greenhouse gas emissions at the point of use hardly renders it a clean and sustainable fuel, and as an energy carrier it is, for several reasons, a poor choice.

Now let us imagine a more sensible future, one in which a carbon-free energy source generates easily transported hydrocarbon fuels, using water and carbon dioxide as chemical inputs. For example, a chemical process might generate alcohols via artificial photosynthesis. Clean and efficient use of that fuel can, in principle, be achieved using solid oxide fuel cells, which offer about twice the efficiency of combustion engines while producing zero emissions of regulated pollutants.

But solid oxide fuel cells don't normally work well when operated directly on hydrocarbon fuels. The culprit is the nickel in the anode, the very element used to catalyse electrochemical conversion of fuels. When hydrocarbon fuels are used, nickel-containing electrodes tend to become clogged with carbon, a process known as 'coking'.

Some progress has been made in finding nickel-free electrode compositions, but the power densities achieved from such reformulated fuel cells have remained low — until this year.

In April, researchers at the University of Texas, Austin, reported that the double perovskite $\text{Sr}_2\text{MgMoO}_{6-\delta}$ shows exceptional activity for electrochemical conversion of methane (Y.-H. Huang *et al. Science* **312**, 254–257; 2006). This gives me hope that, with greater attention to alternative anodes, our world can reach the goal of sustainable energy.

NEWS

Stem cells derived from 'dead' human embryo

It has no brain, heart or nervous system. So how can researchers tell when an embryo has died? That question is likely to become a focus of debate, as scientists search for ways to create 'ethical' human embryonic stem cells.

Many researchers are investigating methods for deriving such cells without destroying human embryos. Now, one group claims to have developed human embryonic stem cells from a very young embryo that had already died.

Miodrag Stojković, who led the project at the University of Newcastle upon Tyne, UK, believes his technique may be acceptable to governments in countries such as Germany and the United States. Laws in these countries restrict the use of embryonic stem-cell lines owing to concerns about killing potential humans.

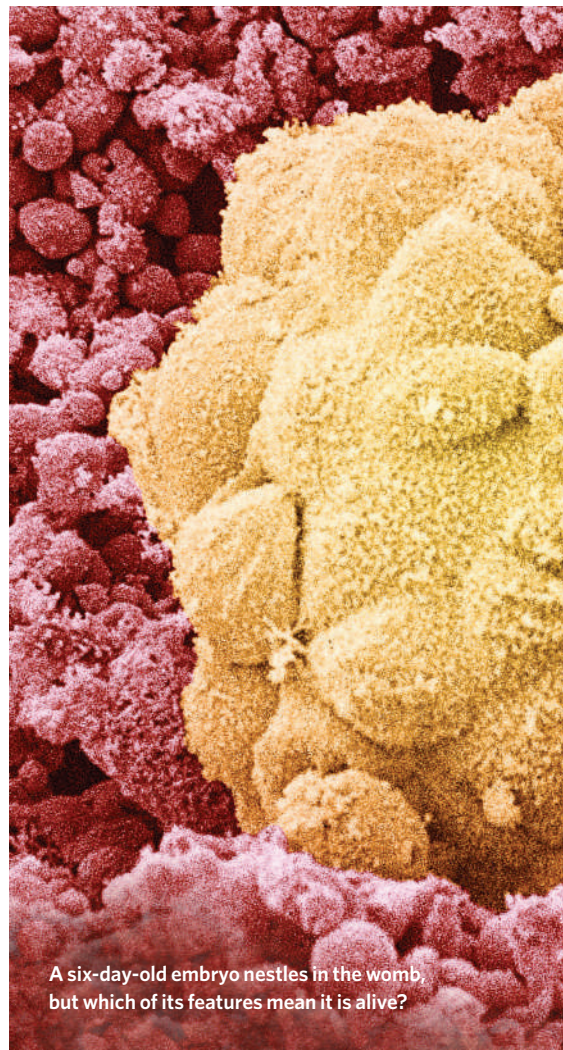
Whether or not the method is judged as harmless will probably depend on whether the lives of such embryos have indisputably ended. "It will intensify the debate over how to define the death of an embryo, because now there is more at stake," says Thomas Murray, president of the Hastings Center, a bioethics research institute in Garrison, New York.

During *in vitro* fertilization (IVF), well over half of human embryos arrest (stop dividing naturally), and are therefore unsuitable for transfer into women. Stojković, now a deputy

director of the Prince Felipe Research Centre in Valencia, Spain, used 161 embryos donated by women in two local IVF clinics in his study, published online last week (X. Zhang *et al. Stem Cells* doi:10.1634/stemcells.2006-0377; 2006). Of these embryos, 29 were developing normally until cells were extracted, 119 arrested 3–5 days after fertilization, and 13 arrested 6–7 days after fertilization.

Arrested embryos were monitored for 24 to 48 hours, to check that none of the cells they contained started dividing again. In an IVF clinic, such embryos would meet the standard embryologist's criteria for having terminally arrested, and would typically be thrown away. Even in Italy, which has particularly restrictive laws stating that embryos cannot be destroyed and that all embryos created during IVF must be implanted, embryos which have arrested in this way are discarded.

The team derived healthy human embryonic stem-cell lines from eight of the normally developing embryos, one of the late-arrested embryos and none of the early-arrested embryos. Although many of the cells in the arrested embryos had distorted shapes or damaged chromosomes, a few cells remained healthy. To coax these into growing, the researchers removed the 'zona pellucida' that sheaths the embryo, and



A six-day-old embryo nestles in the womb, but which of its features mean it is alive?

Superconductivity research is down but not out

"Reports of my death have been greatly exaggerated," Mark Twain allegedly said after his obituary was published prematurely. High-temperature superconductivity physicists now know how he felt.

If current trends continue, research into high-temperature superconductivity (high T_c) will come to a standstill some time between 2010 and 2015, according to a report by Andreas Barth and Werner Marx, respectively at FIZ Karlsruhe and the Max Planck Institute for Solid State Research in Stuttgart, Germany. That news hasn't gone down well.

Barth and Marx scoured

databases owned by the Chemical Abstracts Services (CAS) and the physics and engineering abstracts service Inspec to find how interest in the field was holding up. They searched for all papers published and patents granted until the end of 2005 that involve alkaline earth cuprates containing rare earth elements. These materials show extremely low resistance to electricity at temperatures up to around -200°C . This is much warmer than temperatures normally associated with superconductors. But nobody has been able to work out how such high-temperature

superconductors work.

Barth and Marx plotted the falling number of publications over time, then extrapolated the results to predict that the number of publications on these compounds will drop to zero between 2010 and 2015 "if no ground-breaking discoveries happen to occur".

Superconductivity scientists admit the field has slowed since the flurry of research that followed Georg Bednorz and Alex Müller's 1987 Nobel prize for their discovery of ceramic high-temperature superconductors. "There was a gold rush," says Jan Zaanen of Leiden University, the Netherlands. "It's a

very deep problem — all the easy things have been done."

"All fields of condensed-matter physics undergo periods of ebb and flow," says Paul Grant, who has worked at IBM on high-temperature superconductors. "The general field of superconductivity is wide open."

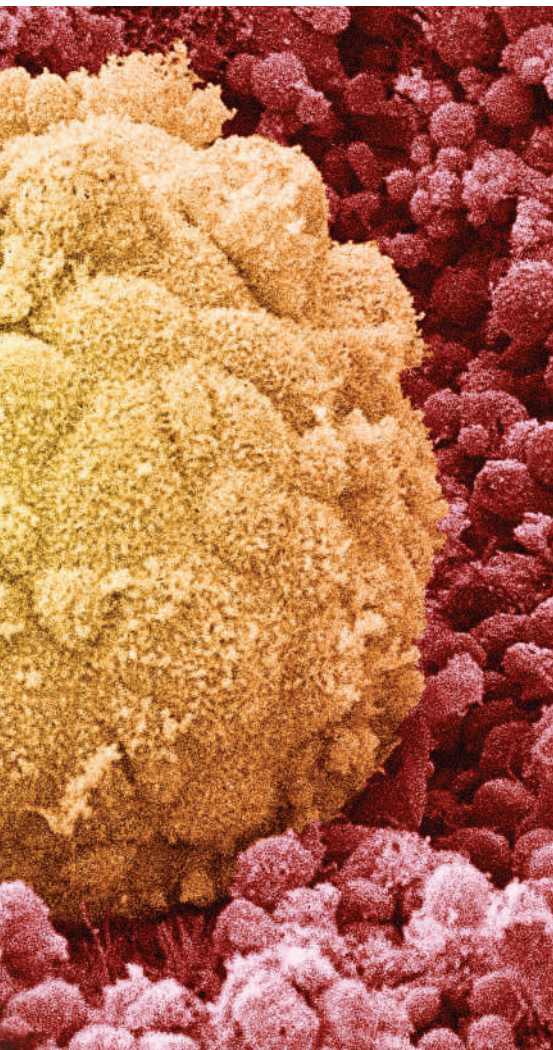
After high-temperature superconductivity was discovered there was a huge spike in publications. Dreams of creating levitating trains, making a fortune and getting a Nobel prize for finding out how these systems work kept the field alive for a while. But as problems remained unsolved, people started to leave. "High T_c made me



BLOGGERS RALLY OVER 'TRIPOLI SIX'

Follow the story of the medics in Libya accused of infecting children with HIV.
www.nature.com/news

F. ABRAHAM/HRV
 Y. NIKAS/WELLCOME PHOTO LIBRARY



used different culture conditions.

The cell line from the arrested embryo seems to behave normally and can generate other tissue types, although "scientists will have lingering doubts about the 'health' of the cell line, if it's derived from a poor-quality embryo," cautions stem-cell researcher George Daley of the Children's Hospital Boston in Massachusetts.

Some stem-cell scientists are excited by Stojković's achievement. "There is no destruction of an embryo involved," says Hans Schöler, a director at the Max Planck Institute for Molecular Biomedicine in Münster, Germany. "If everything is confirmed, I don't see how anyone could attack such cell lines as unethical."

But others say the technique will be dogged by ambiguity over what constitutes a dead embryo. In theory, the approach is similar to harvesting organs from dead people — but in the latter case there are clearly defined criteria for proclaiming someone irreversibly brain dead. There are no such rules for an embryo. And even the remote possibility that an embryo could revive would make this procedure unacceptable to some.

Donald Landry at Columbia University Medical Center, New York, is working to establish "ironclad" criteria to define embryo death. He and co-workers have observed 444 arrested human embryos; of these, 142 had fewer cells at the five-day mark and never developed into a normal blastocyst (D. W. Landry *et al. Regen. Med.* 1, 367–371; 2006). "They were truly dead," he says. Landry is now planning to study embryo death at other stages of development

"I don't see how anyone could attack such cell lines as unethical."

and to find molecules that are only produced by terminally arrested embryos.

Stojković's technique is just the latest proposal for creating 'ethical' human embryonic stem cells. A recent *Nature* paper by Robert Lanza and colleagues from Advanced Cell Technology in Worcester, Massachusetts, sparked criticism after claiming to have made two such lines by taking single cells from embryos; they proved the principle, but did in fact destroy the embryos they used. Earlier this month, the body that operates the US National

Stem Cell Bank agreed to distribute Lanza-style cell lines — if the federal government will fund research on them.

But without a change in current restrictions, most US scientists are unlikely to get their hands on 'ethical' lines. Legislation known as the Dickey Amendment prevents federal money from being spent on research that harms human embryos. Even if new techniques are deemed to leap this hurdle, President George W. Bush has banned federally funded studies on any human embryonic stem-cell lines derived after 9 August 2001.

James Battey, who heads the National Institutes of Health (NIH) Stem Cells Task Force, says the institutes won't seek legal advice on whether new lines could be funded until they receive a grant application for such work. But Landry hopes that embryonic stem cells derived from dead embryos might finally persuade the Bush administration to change its mind. "I think it would pass muster," he says.

Helen Pearson and Alison Abbott

feel old," says Zaanen. Only the diehards remained, he says, some of whom acquired reputations for having large egos and even making superconductivity something of a religion. Zaanen claims those egos have been toned down but says the field is still too intimidating to attract young blood.

Barth and Marx did not include

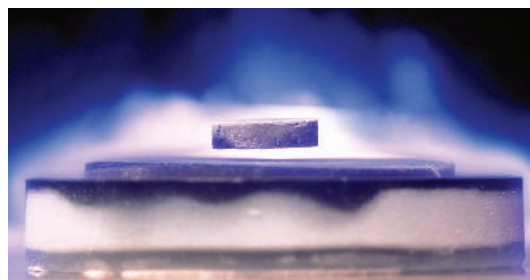
such influences as these in their study. Marx says his aim was to "demonstrate the potential of databases and search systems for generating meta-information that could be interesting in a specific field". As a piece of scientometrics the work is robust, according to Paul Peters, who works at the CAS: "Given the fact that multiple

sources and different approaches all indicate the same demise, I would think this is quite accurate."

But he warns: "Science may prove them wrong." There are signs that high-temperature superconductivity could be having a resurgence. The US Department of Energy's basic energy sciences office recently ran a workshop on superconductivity. Pat Dehmer, the office's associate director, says: "Funding will be very seriously considered for this field," although she remains coy about revealing details before the federal budget announcement in February. A demonstration project using high-temperature superconductor wires in the US national energy grid is also starting this year.

Marx agrees that the field could revive given a breakthrough such as the discovery of superconductors that work at even higher temperatures or are in a new class of materials, or the development of a theoretical explanation of the phenomenon. He also admits that his study has limitations. "Extrapolations are always problematic," he says. "The word 'zero' is perhaps not an optimal expression here. It may be taken seriously but certainly not literally."

That doesn't stop some from taking the conclusion personally. The extrapolation is "utter nonsense" according to Zaanen. "It's a vivid illustration of the blindness of bean counting." **Katharine Sanderson**



A magnet levitates above a ceramic superconductor cooled by liquid nitrogen.

D. PARKER/IMV/UNIV. BIRMINGHAM HIGH TC CONSORTIUM/SPL

Is US hurricane report being quashed?

A statement on the science behind the politically sensitive issue of hurricane activity and climate change has been blocked by officials at the US Department of Commerce, *Nature* has learned.

Work on the statement began this February after complaints about the actions of political appointees at the National Oceanic and Atmospheric Administration (NOAA), an agency that falls under commerce-department control. NOAA researchers accused the appointees of ignoring — on the agency's website and at press conferences — the possibility that global warming could cause hurricanes to become more intense or frequent. The agency was also accused of preventing scientists who believe there might be such a link from speaking out (see *Nature* 439, 896–897; 2006).

The link is a sensitive issue because of the devastation caused by Hurricane Katrina and the US government's reluctance to restrict the greenhouse-gas emissions that are driving climate change.

NOAA officials denied both of the accusations from researchers. But e-mails obtained under freedom-of-information legislation by the environmental group Greenpeace USA, based in Washington DC, show that several NOAA scientists told their seniors that the agency was not properly representing hurricane science. The scientists' complaints prompted the creation of an internal seven-member panel charged with preparing a consensus statement on the views of NOAA researchers on hurricane science. A draft seen by *Nature* states that global warming may be contributing to hurricane

intensity and that further research is needed to clarify the issue.

The document was finalized by the panel in mid-May and was due to be released to the public and the media in time for the start of this year's hurricane season in June. But panel chair Ants Leetmaa, director of NOAA's Geophysical Fluid Dynamics Laboratory at Princeton University, New Jersey, received an e-mail on 18 May from a commerce-department official informing him that the document needed to be made less technical and was not to be released. Leetmaa says department officials have not responded to his efforts to contact them since.

Getting exercised

When asked about the document, NOAA administrator Conrad Lautenbacher told *Nature* that it was simply an internal exercise designed to get researchers to respect each other's points of view. He said it could not be released because the agency cannot take an official position on a field of science that is changing so rapidly. But panel members contacted by *Nature*, including Leetmaa, disagree strongly with this interpretation. Internal NOAA and commerce-department e-mails also discuss the timetable for the document being "cleared" for "distribution". The draft states that it refers to the "current state of the science" and does not contain "any statements of policy or positions of NOAA".

NOAA's stance on hurricane science came in for further criticism last week. Details have

emerged of what critics interpret as government attempts to control media access to Tom Knutson, a NOAA researcher who has published papers suggesting that global warming may make Atlantic hurricanes more intense. E-mails obtained by the online news magazine *Salon* show that Chuck Fuqua, a press officer at the Department of Commerce, enquired about Knutson's views on global warming when considering an interview request from CNBC television. He then asked whether other NOAA researchers who dispute the warming link might be interviewed instead. One such researcher, Chris Landsea, is described in another e-mail as the "best source" to explain why climate change is not increasing hurricane activity.

Lautenbacher says the agency would not "condone anything that works in that way" and that he has taken steps to ensure that researchers are not prevented from talking to the press.

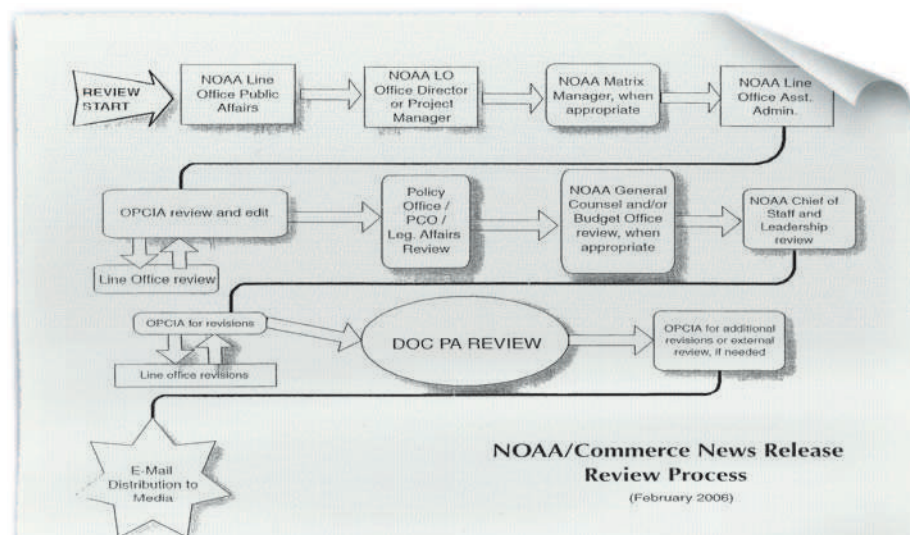
"Despite the blocked consensus report, it does now seem easier for NOAA scientists to speak to the media."

Despite the blocked consensus report, it does now seem easier for scientists to speak to the media. A year or so ago, NOAA researchers complained of having to run interview requests by press officers, but scientists at the agency told *Nature* they are

no longer required to do so. The documents obtained by Greenpeace do, however, indicate that NOAA press releases must still be seen by commerce-department officials as part of a 13-step approval process (pictured below). A department spokesperson says they need to see the releases so that they can prepare for enquiries, adding that officials would never change the scientific content of a release for non-scientific reasons.

Another of the complaints made earlier this year by NOAA researchers has also prompted an agency response, albeit a minor one. According to the Greenpeace e-mails, Knutson and colleagues complained about an article on NOAA's website, which claimed that the recent increase in Atlantic hurricane activity "is not related to greenhouse warming". The article, published last November, stated that there was a "consensus" in the NOAA that the intense 2005 hurricane season was due to natural variability. It was criticized by climate researchers, some of whom believe that global warming may have played a role in recent hurricane activity. The article itself remains unchanged, but the complaint did prompt the addition of a note at the end, stating that it does not represent the views of all NOAA researchers. ■

Jim Giles



Paper trail: this flow chart for press releases was obtained under freedom-of-information legislation.

**MARS IN FOCUS**

For updates on Mars missions, visit our website.
www.nature.com/news/infocus/mars.html

SNAPSHOT**Face facts**

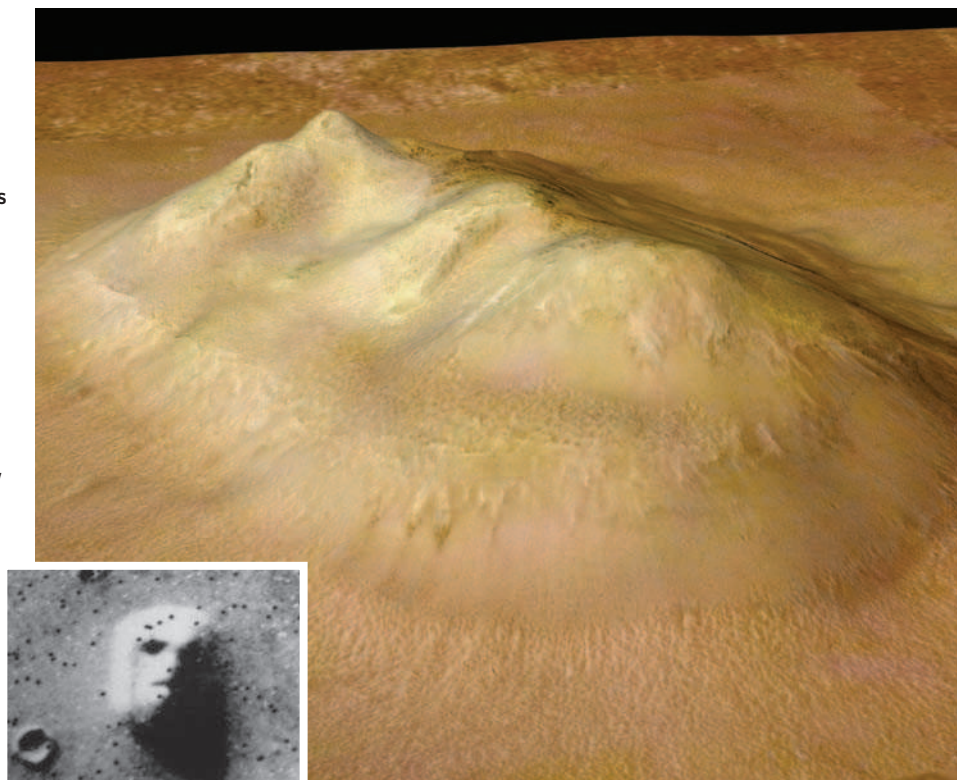
In a picture of Mars taken by the Viking 1 orbiter in 1976 (inset), sunlight caught this hill in a way that it made it look like a shadowy face. Here, the European craft Mars Express reveals it is nothing more than a lumpy rock — as scientists argued all along.

In 1998 and 2001, NASA's Mars Global Surveyor took high-resolution pictures of the outcrop, but failed to quell speculation by some that the feature was a relic of a martian civilization.

Gerhard Neukum, principal investigator for the Mars Express camera, says he received hundreds of e-mails asking for new pictures. "Some of them even said 'NASA is lying, we want the truth'." He says the conspiracy theorists went quiet after the release of the image shown here, constructed using data gathered on 29 December 2004 and 22 July 2006.

The hill, imaged with a resolution of 13.7 metres per pixel, is a few kilometres wide and was probably shaped by erosion.

Jenny Hogan



ESA/DLR/FU BERLIN/MSSS

NASA/JPL

Statistical flaw trips up study of bad stats

When two Spanish researchers checked the statistics in scientific papers from the *BMJ* and *Nature* in 2004, their results prompted a flurry of headlines and soul-searching for editors.

"Sloppy stats shame science" ran the headline in *The Economist*. "Statistical flaws revealed in top journals' papers" declared *New Scientist*. The revelation that more than a third of all *Nature* papers in 2001 contained statistical errors prompted the journal to introduce new checks on quality. At the *BMJ* (formerly the *British Medical Journal*), where one in four papers was found to contain flaws, editors ran an editorial discussing potential solutions.

Now it seems that another manuscript can be added to the pile of flawed publications: the paper by the Spanish researchers. According to an analysis published earlier this month, one of its two conclusions is invalid because it is based on inappropriate statistics. "Statistical tests should be used correctly," notes the dry conclusion of the new paper.

The test in question was employed by ecologists Emili García-Berthou and Carles Alcaraz from the University of Girona to assess the use of rounding. If rounded correctly, the final digit of a number quoted to three or four significant

figures should have an equal chance of being any digit between zero and nine. García-Berthou and Alcaraz found that fours and nines appeared less frequently than expected, perhaps because researchers have an unconscious preference for rounding to five and zero, respectively (E. García-Berthou and C. Alcaraz *BMC Med. Res. Methodol.* **4**, 13; 2004).

But Monwhea Jeng, a physicist at Syracuse University in New York, points out that García-Berthou and Alcaraz employed the Kolmogorov-Smirnov test, which is used to evaluate whether the probability distributions underlying two sets of numbers differ. Jeng says this was a mistake, because the test assumes that the distributions involved are continuous; in the case of the final digit of a number, the distribution is discrete. He says a more appropriate analysis using a chi-squared test shows no evidence of rounding errors (M. Jeng *BMC Med. Res. Methodol.* **6**, 45; 2006).

Two independent statisticians contacted by *Nature* say Jeng's analysis seems to be correct, and a referee report, posted on the journal's website, indicates that use of a different statistical package confirmed Jeng's conclusion.

But García-Berthou insists his use of the test was appropriate, and says he will explain why in a letter to the journal. The argument could be viewed as no more than amusingly ironic, especially because Jeng's analysis does not affect García-Berthou and Alcaraz's second and perhaps more important discovery: that many *P* values in *Nature* and *BMJ* papers were wrongly calculated. But the incident does illustrate the difficulty of assessing whether statistical tests have been properly used, especially given that scientists well trained in statistics do not always agree.

"If a journal doesn't have enough expertise it's a real problem," says Steve Goodman, a medical statistician and editor at the *Annals of Internal Medicine*. He says he rarely sees papers that are free of flaws. "I view most of the literature as done wrong."

A study published this July, for example, examines the use of *P* values in paper abstracts (P. C. Göttsche *BMJ* **333**, 231–234; 2006). The paper concludes: "Significant results in abstracts are common but should generally be disbelieved."

Jim Giles

"I view most of the literature as done wrong."

SCORECARD

Saving the planet
NASA's planetary protection officer, John Rummel, is leaving. His two main tasks: protecting other planets from contamination by us, and protecting us from alien life. Seems he had a perfect record.

Golden age

Entrepreneur Peter Thiel has pledged US\$3.5 million to researcher Aubrey de Grey to pursue his theory that people can live indefinitely. But de Grey reckons he needs \$1 billion.

ZOO NEWS

Cosy penguins

Up to 2,000 fibreglass igloos are being built to house endangered penguins on Dyer Island in South Africa. But it's not ice the birds are missing — it's guano. The penguins use it for nests, but since the 1970s humans have been harvesting it for fertilizer.

Chainsaw reaction

In February, a US agency announced that it was mapping the habitat of an endangered woodpecker around Boiling Spring Lakes in North Carolina. The move could subject areas of the town to more stringent building restrictions. Landowners have responded by felling thousands of trees to drive the woodpecker out.

ON THE RECORD

"It won't stop until some of these scientists are dead."

Climatologist James Hansen sees a slow end to climate scepticism.

NUMBER CRUNCH

14 shuttle flights are needed in the next four years to finish the International Space Station.

19 shuttle flights were achieved in the four years between 1988 and 1991, when NASA also had three operational shuttles.

3 shuttle flights have taken place in the past three years.

Sources: *San Francisco Chronicle*, *Planet Ark*, *The New York Times*

Bad Boys question received wisdom on HIV

HIV is a frustrating foe, but at least doctors have been able to count on a few solid principles to help them fight the virus. Now, a group of scientists calling itself 'The Bad Boys of Cleveland' reports evidence that rebels against one of those principles. The findings cement a feeling that has been growing in the HIV research community: that the virus enlists patients' own defences to dismantle their immune systems.

This revises the picture first painted a decade ago, when studies reported that levels of HIV in a patient's blood predict how fast the patient will lose vital T cells. Since then, doctors have believed that the virus is the main trigger for T-cell loss. They rely on measurements of viral load to help them decide when and how to treat patients with HIV.

But it is increasingly clear that virus levels are only a small part of the story. In fact, as Benigno Rodriguez of Case Western Reserve University in Cleveland reports this week, viral load explains only 4–6% of the rate at which a

patient's T cells disappear (B. Rodriguez *et al.* *J. Am. Med. Assoc.* **296**, 1498–1506; 2006).

Rodriguez and his collaborators in California, Massachusetts and Washington examined blood samples from thousands of patients dating from 1984. They analysed the patients' viral load and T-cell count before treatment with antiretroviral drugs. The group found that, in general, patients with higher viral loads lost their T cells faster than those with lower virus levels. But the disease progressed at different rates in patients with similar viral loads. This suggests that something other than virus levels alone is driving T-cell loss.

Now, scientists must find out what that is. Many suspect that a phenomenon called immune activation is an important factor. This is the idea that HIV whips the body's immune system into a frenzy, and that this flurry of activity eventually triggers T-cell death. "This paper will help shift the focus of basic research to immune activation," says immunologist Zvi Grossman of the US National Institutes of

"We've been doing this on a shoestring — people pay their own air fare. They come because they like the atmosphere."

Mouse brain map is complete

Three years, 21,000 genes and US\$41 million after the Allen Brain Atlas was begun, it is finished. A three-dimensional map of gene expression in the mouse brain, the atlas is the most comprehensive study of its kind to date.

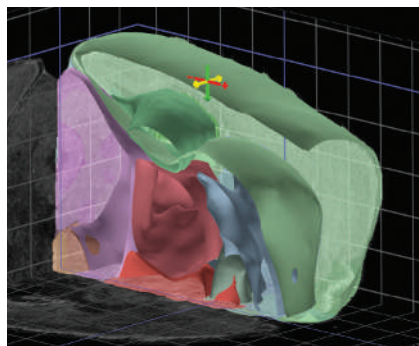
A personal interest in neuroscience saw Paul Allen, the billionaire philanthropist who co-founded Microsoft, establish the Allen Institute for Brain Science in Seattle, Washington, in 2001. He donated \$100 million: half went on the Brain Atlas, launched in 2003 as the institute's first major project after two years of consultation.

Researchers mapped where each of the mouse

brain's 21,000 genes are expressed by staining brain sections with probes specific to each gene. The resulting atlas provides insight into the brain's function, helping researchers understand how different regions operate and interact. Allan Jones, chief

scientific officer at the Allen Institute, is confident that the atlas will "dramatically propel neuroscience forwards".

The atlas is available free of charge online in the form of a database. Users can search for particular genes, then scroll through



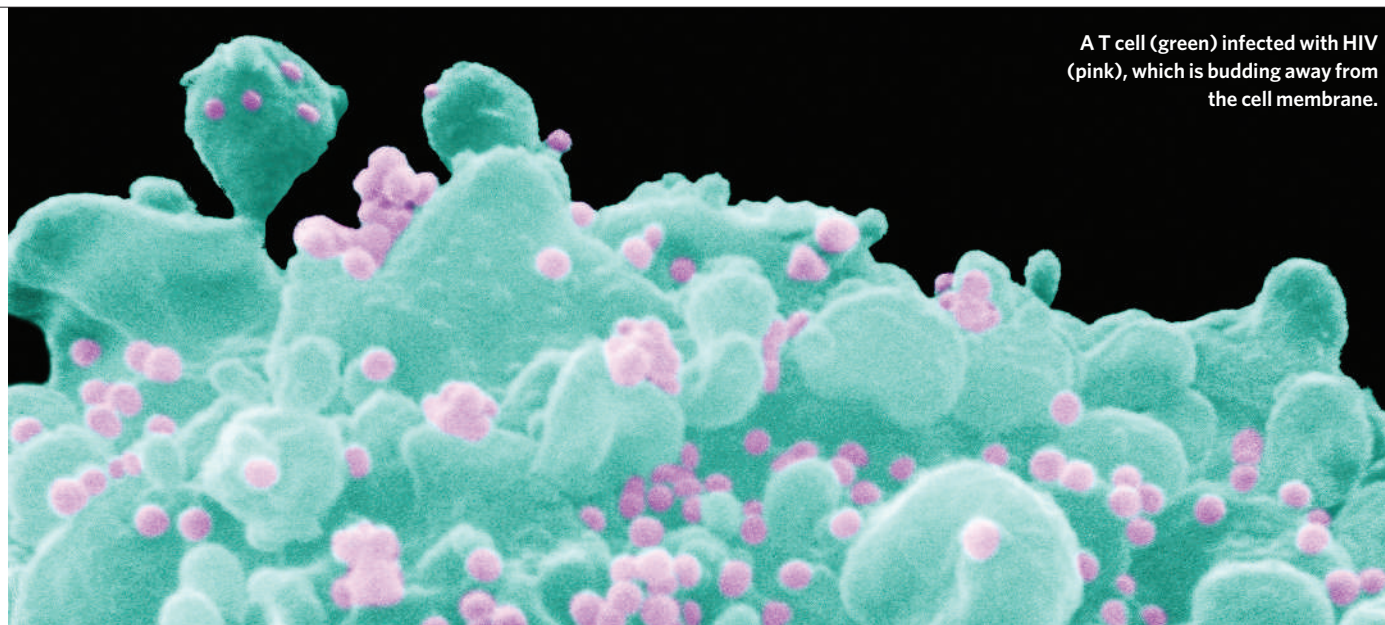
Computer-generated cutaway of a mouse brain from Allen Brain Atlas data.

ALLEN INSTITUTE FOR BRAIN SCIENCE



INTO ORBIT FOR LESS
Just how cheap can
spaceflight get?
www.nature.com/news

NIBSC/SPL CU SPACEFLIGHT



A T cell (green) infected with HIV (pink), which is budding away from the cell membrane.

Health in Bethesda, Maryland.

The paper is also a score for the Bad Boys — a group of immunologists who have been meeting informally over the past two years. They include about 30 scientists, including Grossman, of whom a handful are 'Bad Girls'.

The Bad Boys were first convened by Michael Lederman, head of the Case Western Reserve University Center for AIDS Research and senior author of the recent paper. "The idea was to have a relaxed but intense forum where

we could present our unpublished data, share ideas, then go back to our lives and work some more," Lederman says. "We've been doing this on a shoestring — people pay their own air fare. They come because they like the atmosphere in which we work."

Many see that informal approach as a more refreshing form of collaboration than the large, top-down structures fashionable in HIV research these days (see *Nature* **442**, 610–611; 2006). But in their fight against orthodoxy, the

Bad Boys have made one concession to scientific propriety. The *Journal of the American Medical Association* paper is the first to reference the Bad Boys, but it acknowledges the group by a much blander name: the Cleveland Immunopathogenesis Consortium. Lederman admits that the wordy title "lacks panache" compared with the Bad Boys nickname. But, he says: "We were anxious that editors might give us grief."

Erika Check

photographs of vertical or horizontal sections to see how expression is distributed. The completed atlas contains around 85 million images and 600 terabytes of data — enough to fill more than 7,500 top-of-the-range iPods.

The mice used in the Allen Brain Atlas were all 56 days old. An alternative project, the Gene Expression Nervous System Atlas (GENSAT), launched by the US National Institutes of Health in 2003, is mapping gene expression in the mouse brain throughout development. The project has begun to publish results but is still some way from completion.

Much of the Allen Brain Atlas information was released before the project was completed, and researchers in the field already seem pleased with the resource. According to the Allen Institute for

Brain Science, 250 scientists are using the atlas each day, and it is turning up in citations. "Combining the classical approaches of brain research with this new genetic approach is a breakthrough in neuroscience," says Susumu Tonegawa, director of the Picower Center for Learning and Memory at the Massachusetts Institute of Technology. "It's an extremely powerful approach to try to understand the brain."

"This will likely become the reference atlas for molecular and cellular neuroscientists," adds Thomas Insel, director of the National Institute of Mental Health in Bethesda, Maryland. He says the atlas has already produced two surprising results: "First, the percentage of genes expressed

in the adult brain is greater than we had expected, and second, the regional distribution is more selective than expected."

Alongside data on gene expression, the atlas provides an anatomical map of the mouse brain. Charles Watson is an expert on

brain mapping, currently working with George Paxinos to produce the sixth edition of *The*

Rat Brain in Stereotaxic Coordinates — one of the most cited texts in the field. "The project is doing a really good thing and it's fantastic that someone has invested so much money in neuroscience," he says.

But Watson notes that the makers of the mouse atlas have chosen a different set of abbreviations from those in Paxinos' books. "We have the

lingua franca and they're refusing to use it, which seems a bit silly," he says.

Mice are an important model species in neuroscience research, due in part to their potential for genetic manipulation and drug testing not possible in humans. A corresponding atlas of the human brain is an obvious next step, however, and the Allen Institute has initiated pilot studies towards this end. "We plan to address key questions about the human brain and will be focusing our internal research efforts on understanding the cortex — the part of the brain associated with 'higher order' functions," says Jones.

David Brill

Nature has a commercial collaboration with the Allen Brain Institute in the Neuroscience Gateway.

► www.brainatlas.org/aba

'Big science' protein project under fire

TOKYO

It was to have been a giant step towards understanding the many different ways in which a protein can fold. That knowledge would enable one to predict the characteristic structure of any given protein from its gene sequence — a critical tool for understanding how proteins interact and for developing effective drugs.

But Japan's ambitious Protein 3000 project will end its five-year term next March amid criticism that, despite looking set to meet its goal of solving 3,000 structures, the information gained is of limited use, and its high-throughput approach might even be depriving the field of valuable research skills. Recent science-ministry budget requests show that protein research in Japan will change dramatically, focusing on new analysis methods and on proteins related to specific diseases. The shift means an uncertain fate for the huge nuclear magnetic resonance (NMR) facility that has been the backbone of the project.

Protein structures are mainly determined by two methods — NMR and X-ray crystallography. NMR detects the magnetic properties of the atoms in proteins in solution to identify how they are arranged. X-ray crystallography requires that proteins first be crystallized. But many biologically important proteins are too big for NMR and impossible to crystallize.

Instead, the five-year project aimed to develop a 'reference library' of representative protein folds. Scientists could then translate information about the amino-acid building-blocks into predictions about overall structure, even of larger molecules. The project has received roughly ¥8 billion (US\$70 million) per year since April 2002. Half of that money went to the RIKEN research institute to solve 2,500 structures using its NMR facility in Yokohama and its X-ray beamlines at the SPring-8 synchrotron radiation facility in Harima. The other half went to labs targeting specific proteins.

The project has, in quantitative terms, sailed along. Japan has an impressive 40 NMR machines, and Shigeyuki Yokoyama, who heads the NMR facility and is overall director of the project, says that by March this year the two RIKEN facilities had produced nearly 1,600 structures, with the project as a whole set to meet its goal of 3,000 by next March. "In this sense it is a great success," says Kurt Wüthrich of ETH Zurich, who won the 2002 Nobel chemistry prize for developing NMR to determine protein structures.



RIKEN YOKOHAMA INST.

Protein-structure work performed at the RIKEN NMR facility in Yokohama has not impressed everyone.

But big numbers aren't enough, say vocal critics in Japan. "A lot of it is junk," says Masatsune Kainosho of Tokyo Metropolitan University, reflecting the sentiment of many protein researchers contacted by *Nature*. He says a lot of the structures solved so far are similar, and relatively easy: "They've gone after a lot of low-hanging fruit." The criticism is compounded by a shifting benchmark. Japan's contribution of 3,000 protein structures was supposed to form a third of about 10,000 protein folds thought to exist. That number has now jumped to 16,000 by Yokoyama's estimation and may be 30,000 according to others.

Several researchers have also expressed concern that the factory approach at the NMR facility has deprived young researchers there of the skills necessary to solve more complicated and important scientific riddles. It might have "destroyed the next generation," says one.

Wüthrich, who helped plan the NMR centre in 1998 and was a science adviser in 2000–04, agrees that the facility is a wasted opportunity. "A centre of that size should contribute to methodology, but there has been nothing," he says. "It became a one-man show with 40 NMR machines — there is no knowledge."

Yokoyama defends his project, pointing out that Protein 3000 has cracked the structure of many signal-transduction proteins involved in disease. Supporters on the project add that it is too early to judge the value of the structures.

Yokoyama points to a long list of publications and patents related to the project, but admits: "We were too busy solving structures, and didn't have time to advertise the function."

Meanwhile, budget requests from the science ministry last month show a shift away from high-throughput methods, reflecting a change of policy. From next April, ¥5 billion has been requested to develop new methods for analysing and manipulating proteins, as well as new information technologies to present and disseminate the collected data. Another ¥2.4 billion has been requested to analyse specific disease-related proteins.

Feasibility studies on candidate projects for next year will start next month, and will include a study led by Kainosho on a new method to apply NMR to larger proteins (see *Nature* **400**, 52–57; 2006) and an exploration, led by the High Energy Accelerator Research Organization, of a microfocus X-ray beam that allows the analysis of smaller crystals.

Yokoyama had hoped to continue at full speed, but now expects to lose as much as half of his previous budget for the NMR facility. "It's a very difficult situation for me and for the people working on the project," he says. To help keep his scientists busy, Yokoyama says he will seek new users from industry and from other countries. He is also working with scientists in Korea to make the NMR machines available to Asian countries that would otherwise have no access to such cutting-edge technology. ■

David Cyranoski

"It became a one-man show with 40 NMR machines — there is no knowledge."

Japanese researcher admits fabricating data

Osaka University researcher Akio Sugino acted alone in manipulating data for two papers in the *Journal of Biological Chemistry*, according to a report from the university's ethics committee last week.

The committee found no evidence linking the misconduct to the suicide of Yasuo Kawasaki, an assistant professor who helped blow the whistle on Sugino (see *Nature* 443, 253; 2006). But Sugino admits fabricating data for one of the articles, now retracted (W. Nakai *et al. J. Biol. Chem.* 10.1074/jbc.M603586200; 2006), saying he was rushing to meet a deadline for a student's grant application. According to the report, he claims that he planned to replace the data during the editorial process.

Sugino also admits there is confusion concerning data in the second paper (T. Seki *et al. J. Biol. Chem.* 281, 21422–21432; 2006), but claims he has original data to back it up. His co-authors have asked for a retraction.

Osaka University is now considering what disciplinary action to take. The 62-year-old Sugino is set to retire next March.

Tycoon gives \$3 billion to climate-change initiatives

Is it philanthropy or a shrewd business decision? Richard Branson, the flamboyant British entrepreneur, has pledged to invest all profits from his transport businesses, including the Virgin Atlantic airline and Virgin Trains, into climate-change initiatives. The total is estimated to be US\$3 billion over the next 10 years.

Branson made the pledge at the Clinton Global Initiative conference in New York last week. He has already invested in Cilion, a California-based bioethanol company. And Virgin Fuels will put \$400 million over three years into biofuels investment and research.

The conference also saw promises from pharmaceutical company Pfizer, which has pledged \$15 million over five years for malaria research, and the International



Going green? Richard Branson will use profits from his transport businesses to tackle climate change.

Laboratories shape up for the subcellular dance

US artist Mara Haseltine's sculptures are derived from images of viruses and subcellular components obtained by electron microscopy and nuclear magnetic resonance.

The sprawling *Waltz of the Polypeptides*, a section of which is pictured here, will be dedicated at Cold Spring Harbor Laboratory in New York on 7 October. Another work, *SARS Inhibited*, was unveiled at the Biopolis research park in Singapore on 20 September. It represents the active site of a key enzyme in the SARS virus that can form a target for drugs against the disease. The viewer can stand inside the sculpture, becoming, metaphorically, the cure.

Haseltine's father, William, is a well-known geneticist.



M. HASELTINE

AIDS Vaccine Initiative, which has pledged \$2.5 million over three years towards tackling HIV in India.

Report calls for changes to FDA to ensure drug safety

A committee of the US Institute of Medicine last week called for the Food and Drug Administration (FDA) to be given more funding and authority to police the safety of prescription medicines.

The entire FDA is "severely underfunded", the authors write in their sharply worded report, but "resources for postmarketing drug safety work are especially inadequate".

The committee's report, *The Future of Drug Safety*, says drugs should carry a warning label and be subject to advertising restrictions for their first two years on the market. It also says the FDA should be given authority to fine companies that fail to carry out promised safety studies on marketed drugs. The agency requested the study in late 2004, shortly after Merck withdrew the painkiller Vioxx because of evidence that it increased the risk of heart attack and stroke.

But the drug industry's lobby group contests the report, saying fewer than 3% of prescription drugs have been withdrawn for safety reasons over the past two decades.

Cash brings nuclear-fuel stockpile one step closer

A proposed international nuclear-fuel bank became more likely with the announcement last week of a US\$50-million donation to jump-start the project.

The money comes from the Nuclear Threat Initiative in Washington DC, which works to prevent the spread of nuclear weapons. The group's co-chairman, former senator Sam Nunn, made the announcement

in Vienna at a meeting of the International Atomic Energy Agency (IAEA).

The nuclear-fuel bank would supply partly enriched uranium to nations with operational nuclear power plants but no ability to enrich uranium themselves (see *Nature* 438, 268–269; 2005). The hope is to remove the incentive, and the justification, for countries such as Iran to develop their own uranium-enrichment capabilities.

But the pledge is contingent on IAEA member states contributing a further \$100 million, or an equivalent value of partly enriched uranium, and on the IAEA acting within two years to approve the reserve.

Scientist jailed for series of animal-rights attacks

A cancer researcher at the University of Nottingham, UK, has been jailed for three years after pleading guilty to a series of attacks on the property of companies supplying equipment to the animal-research firm Huntingdon Life Sciences.

Joseph Harris is the first animal-rights activist to be convicted under the Serious and Organised Crime Act, introduced in 2005. His lawyer says his career placed him under increasing pressure to conduct animal experiments, which conflicted with his ethical beliefs.

Harris, who is not a member of any animal-rights group, caused damage totalling more than £25,000 (US\$50,000) to three firms whose details he apparently obtained from the Stop Huntingdon Animal Cruelty website.

Correction

The story "That's no laser, it's a particle accelerator" (*Nature* 443, 256; 2006) incorrectly stated that the device described could accelerate electrons to 0.15% of their initial speed. That number actually refers to the change in kinetic energy of the electron bunches.

BUSINESS

DLR



Stay back: wake turbulence could see the Airbus A380 lose take-off and landing slots.

Turbulent times

The bid by Airbus to challenge the Boeing 747's dominance of the market faces an aerodynamic obstacle. **Ned Stafford** reports.

The success of the world's biggest passenger airliner could hinge on an obscure regulatory decision to be made by the International Civil Aviation Organization (ICAO) later this year.

The European firm Airbus hopes that its A380 can break the Boeing 747's stranglehold on the market for very large airliners. But draft guidelines issued late last year for the European challenger suggest that it could be hampered by safety restrictions.

Over the past decade, the European firm has drawn level with Boeing in terms of overall airliner sales, establishing itself as a rare example of European success in a huge, high-technology industry. However, US industrialists and politicians have bitterly complained that its financing arrangements amount to an unfair subsidy by European governments.

In the airliner market, meanwhile, Boeing has begun to reassert itself, once again surpassing Airbus in total sales. The US manufacturer has rejected costly plans to build a super-jumbo akin to the A380, leaving the European manufacturer — and much of Europe's entire aviation industry — relying on the success of the project. But that success is very much in doubt: back in July, Airbus chief executive Gustav Humbert quit over delays to the A380 and other management issues at the company.

If final regulations due this November resemble the preliminary ones, they will add to the project's woes. They will mean that other planes would have to stay farther away from the A380 than from 747s. This could see the A380 lose valuable take-off and landing slots at busy airports, compromising its bid to establish itself in the market.

Bumpy ride

The problem is turbulent airflow. All aircraft create tornado-like counter-rotating vortices that trail from the tips of the wings as they fly. These can pose a safety risk to other aircraft — especially during take-off and landing. Last year, the ICAO shocked Airbus by suggesting that jets set to take off after A380s should wait for 60 seconds longer than they do behind Boeing 747s. It also said that planes landing after an A380 should stay back 18 kilometres — about twice the distance they have to hang back from 747s. And the ICAO suggested more stringent horizontal and vertical spacing requirements for the A380 at cruising altitudes.

Airbus won't comment on the requirements, but Jaakko Hoffren, an aeronautical engineer at the Helsinki University of Technology in Finland says: "My impression is that Airbus had always assumed that the in-flight separation requirements for the A380

would be the same as for the Boeing 747."

Increased spacing requirements would defeat the whole rationale for the A380, he asserts. Most major hub airports already have too few take-off and landing slots. If the preliminary requirements get implemented, each A380 flight would mean fewer slots.

Since the preliminary guidelines were issued, Airbus has commissioned the German Aerospace Agency, the DLR, to conduct research that it hopes will prove the A380 is safe at spacing distances closer to, or the same as, those for the Boeing 747. The research used light detection and ranging (LIDAR) — a technique similar to radar, but using light instead of radio waves — to study the vortices created by airliners. It also flew airliners into the wakes of others at cruising altitude, to compare the levels of turbulence.

These studies were "a worldwide first", says David Voskuhl, a spokesman for Airbus. At cruising altitudes "no difference has been found between the two aircraft types", he says. "At low altitude, there is a slightly bigger vortex on the A380, compared with the 747." Voskuhl adds that it is up to the ICAO to decide safe distances for the new aircraft. Boeing and the ICAO declined to comment.

Increased aircraft weight strengthens wake vortices, whereas longer wingspans and increased speed reduce their intensity. The A380 has a maximum take-off weight of 560 tonnes and wingspan of 80 metres, compared with the 747's 397 tonnes and 64 metres.

Current ICAO guidelines divide commercial aircraft into three weight categories: small, medium and heavy. Thomas Gerz, a meteorologist and head of the aerospace agency's wake-vortex project, says that the preliminary guidelines would create a fourth 'super-heavy-weight' class, with the A380 as its only member. "There is no reason for this," he says. "The A380 does not justify another weight class." He acknowledges that A380s produce stronger vortices than 747s, but contends that current ICAO spacing guidelines are "very conservative".

Hoffren says that his calculations suggest "the in-flight separation limits, in time or distance, behind an A380 should be some 10–30% larger than behind a Boeing 747, for similar hazard levels". He agrees that the ICAO's preliminary A380 guidelines were too stringent — but sees little prospect that the final ones will be the same as those for the 747.

Hoffren predicts that a surprise compromise could be in store when the ICAO announces its final guidelines. According to the grapevine, Hoffren says, the regulator could deem the A380 safe to fly more closely behind other aircraft — on account of its bulk. That would allow it to leave more space in its wake, while occupying the same time slot as its rival. ■

WISHING FOR THE STARS

US astronomers are renowned for getting together and choosing their projects as a group. But just as these tactics are catching on in other fields, some say the astronomy process is grinding to a halt. **Geoff Brumfiel** investigates.

Some of us are impulse buyers, some chaotic shoppers, and some of us head out with a carefully thought through list of what we want. Collectively, US astronomers fit in that last category. Since the 1960s, a who's who of the field has gathered together every ten years to draw up a table of what instruments they want to buy — or rather, to have bought for them. Their reports, known as decadal surveys, cram radio dishes, ground-based telescopes and ambitious satellite observatories into a tidy, page-long, prioritized list.

Compiled through the US National Academy of Sciences, these surveys can do much to set the field's agenda, especially when they prioritize a ground-breaking instrument. The 1964 recommendation to build the 27-dish array that went on to become the Very Large Array in New Mexico led to an explosion in radio astronomy. A 1972 recommendation for research towards the goal of a large space telescope did not, in itself, bring about the birth of the Hubble Space Telescope, but it helped show the community's support during the early years of the project. Cosmic-ray facilities in the 1980s and infrared astronomy in the 1990s both received fruitful bursts of interest and investment thanks in part to earlier decadal surveys.

The astronomers' orderly and concise sorting of their needs has won them the praise of Washington politicians and the imitation of their peers in other disciplines. In recent years, space physics, high-energy physics, atomic and molecular physics, and planetary science have all compiled their own decadal surveys in the hope of galvanizing researchers and winning funding from agencies. Even Earth scientists and botanists have taken a stab at the process. Meanwhile, across the Atlantic, European astronomers are trying to develop a Europe-wide set of priorities (see 'Continental drift', overleaf).

Cloudy skies

But at precisely the time others are rushing to emulate them, the astronomers have run into trouble. It is now five years since the publication of the most recent report, and not one of the instruments it recommended is yet operational. That is not unusual: none of the 1991 report's recommendations was up and running in 1996. And if all goes well, the first of 2001's list — the Gamma-ray Large Area Space Telescope — will launch on 7 October. But in stark contrast with previous surveys, many of 2001's other recommendations have yet to leave the starting blocks and are a long way from being launched or built (see 'Where are they now?', overleaf). Only one other space mission from the survey is likely to fly this decade. The really large space missions are all deferred to the 2010s, as are most of the ground-based projects.

As telescopes and satellites languish in the queue, the designs to which the 2001 report gave its blessing run an increasing risk of

becoming technologically outdated. And the survey's price estimates are proving to be a long way wide of the mark. The cost of its chief priority, a successor to Hubble known as the James Webb Space Telescope (JWST), has gone from \$1 billion to \$4.5 billion (see *Nature* **440**, 140–143; 2006); and the Constellation-X quartet of X-ray telescopes has risen from \$800 million to \$1.6 billion. "I think the last decadal survey for astronomy was a failure," says Webster Cash, an astronomer at the University of Colorado, Boulder. "We basically just have the JWST seven years from now, and until then we're surviving on existing missions. Space astronomy is grinding to a halt."

Get back on track

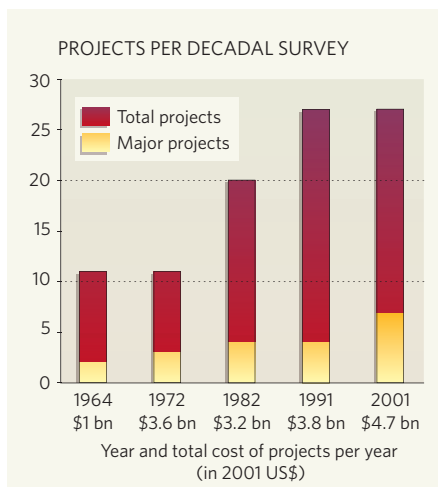
It is against this background of minimal success that the community will begin work on the next decadal review this winter. And as it discusses what should go into the report, it will also be assessing how the surveys can be made to work as they once did. Michael Turner, a cosmologist at the University of Chicago, was until recently head of the National Science Foundation's Mathematical and Physical Sciences Directorate, in which capacity he set up a decadal review for particle physics. "The philosophy of the astronomical decadal survey was 'one blessing with holy water' — you touch a project and by the end of a decade it should be done," he says. In today's environment "that philosophy really doesn't make sense", he adds. Turner is one of several scientists calling for reform of the decadal-review process. He wants to see the survey focus on scientific goals rather than equipment and to give the projects realistic price tags.

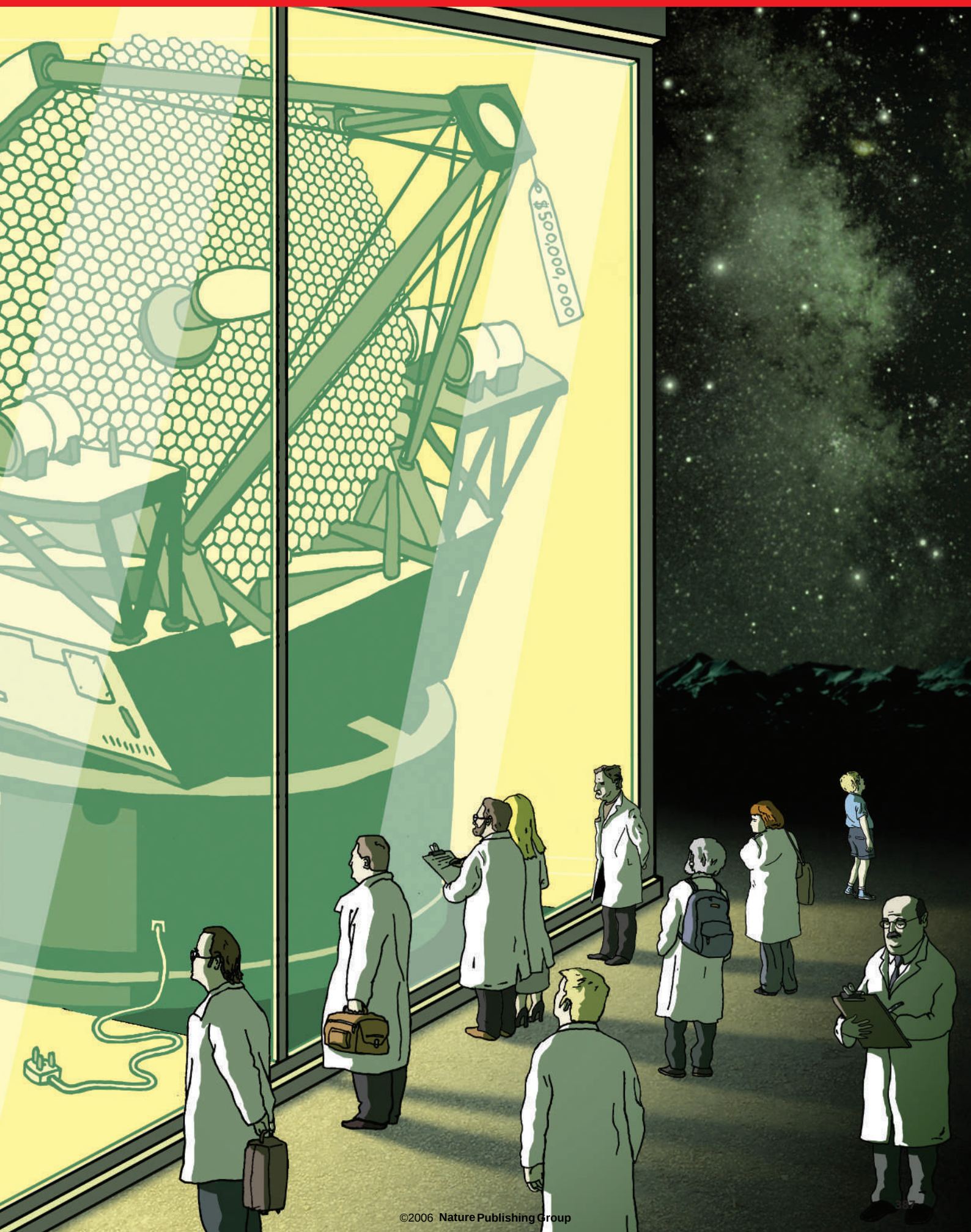
Astronomy has always been an easier discipline to organize than most. "We're all building experiments that go either in space or on the ground, look at the sky and make measurements of distant objects," says Steven Kahn, deputy director of the Kavli Institute for Particle Astrophysics and Cosmology at Stanford University, California. "Most astronomers have some comprehension of what other astronomers are doing. You feel like you can make comparisons or make rankings."

But the surveys are rooted as much in political economy as in epistemology. The first survey was completed in 1964, following efforts to gain a lead in the space race with the Soviet Union. The eight-member panel was convened to work out how best to channel a flood of new funding into ambitious projects that might be beyond individual universities and foundations. Confined to ground-based projects, its priciest recommendation was for a \$40-million 'pencil-beam array' of radio dishes, which eventually became the Very Large Array.

The next review also considered instruments in space, with its 23 authors recommending 11 space-based and ground-based facilities in a growing number of fields, including infrared and high-energy gamma-ray research. Unlike

"The last decadal survey for astronomy was a failure."
— Webster Cash







The GLAST telescope is the only project from the last decadal review that is ready for launch.

the 1964 survey, the 1972 panel ranked the projects. NASA, which funds most space-based projects, has its own planning process that runs in parallel with the decadal reviews, but their priorities often coincide.

Over subsequent decades, the size of the committee grew further, as did the number of projects it recommended (see graph). The most recent survey, completed in 2001, involved more than 100 individuals and was drawn up by a central committee of 15, flanked by 9 subcommittees, each devoted to a separate field of astronomy. It recommended a total of 27 projects, including 7 'major initiatives' — more big ones than any previous decadal review.

Inflation from all angles

The increasing size of the panel reflects the growth of astronomy, says Stephen Strom, an astronomer at the National Optical Astronomy Observatory in Tucson, Arizona, who has served on four of the five decadal reviews. "Astronomy was not a terribly large field when the first of these came out, and it was possible to more or less name the leaders," he says. The process now includes panels, public meetings and white papers from subdisciplines.

The fortunes of subdisciplines can be determined by a nod from a decadal-review committee. For example, the 1991 report chaired by John Bahcall endorsed several major infrared projects. According to Harley Thronson, a chief scientist for exploration at NASA's Goddard Space Flight Center in Greenbelt, Maryland, infrared astronomy was

"There was a tremendous amount of undercosting going on." — Chris Reynolds

"a respectable field" before the 1991 report, but progress was slow. The Infrared Astronomical Satellite, a joint programme between the United States, the Netherlands and Britain, had provided a tantalizing look at long infrared wavelengths in the Galaxy in the early 1980s; but plans for the next US space-based observatory, known today as the Spitzer Space Telescope, were caught up in NASA's bureaucracy. Then the Bahcall committee gave it high marks. "The Bahcall committee gave it the official, highest-level imprimatur," says Thronson. This endorsement, he says "established the credibility, the substance, the importance of observations at long wavelengths".

Making the grade

It's that kind of power that has left astronomers enamoured of the decadal review. "It builds a consensus in the field and it prioritizes," says Turner. "Any group of scientists can produce a wish list that exceeds the GNP of the Milky Way," adds Roger Blandford, director of the Kavli Institute for Particle Astrophysics and Cosmology. The decadal-review process helps astronomers to whittle the list down to a realistic level and "make tough calls".

If the surveys appealed only to scientists they would be of limited value, but they appeal to Washington too. Agencies such as NASA and the National Science Foundation often depend on the survey to help them determine which programmes to back. And the tidy list of priorities makes it a favourite with the White House Office of Management and Budget, which recommends yearly spending for those agencies. It also steadies the United States' famously chaotic congressional budget process, according to David Goldston, chief of staff for the House Committee on Science. "In general, on real science questions, Congress tends to defer to the community," he says. "It's an area where Congress really recognizes that it doesn't have the expertise to make a decision on its own."

The survey's popularity in Washington has led other disciplines to copy it. "A babble of voices is not useful," says Berrien Moore, a climatologist at the University of New Hampshire in Durham, who is co-chairing the Earth science community's first decadal survey. "The community has got to come together and decadal surveys force that." Not all of the surveys are carried out the same way, or necessarily with quite the same ends in mind. The broad field of atomic, molecular and optical science shunned specific projects in favour of endorsing research areas such as physics at ultra-short time periods or ultra-high temperatures. The survey for high-energy physicists, which Turner helped to organize, on the other hand, was geared towards

Continental drift

The popularity of the decadal review extends well beyond the United States, and many European astronomers have their own national survey processes. But these national reviews have yet to be combined into a continental consensus, says Catherine Cesarsky, director-general of the European Southern Observatory (ESO). "We are not yet a united states of Europe."

A consortium of 11 science agencies is trying to do something about that. Known as Astronet

(www.astronet-eu.org), the group wants to build a pan-European list of space missions and telescopes, says Anne-Marie Lagrange, head of the astronomy directorate at the CNRS, France's basic research agency, based in Paris. Unlike the US decadal review, which is coordinated by the community, Astronet has been convened by funding agencies, which frequently collaborate to build European projects. "We felt there was a strong need for a shared science vision for astronomy at the

European level," Lagrange says. "Astronomy tools are getting more and more complex and expensive."

Over the next three years, the group will set up advisory committees and hold public meetings to try to draw up its list. The task may not be as complex as it first seems. The two main funding bodies for much of astronomy — the ESO and the European Space Agency — are already Europe-wide, and many of the national decadal surveys share similar priorities.

But it is nevertheless important to create a master list, especially as the European Union (EU) develops new pan-European funding bodies such as the European Research Council, says Reinhard Genzel, a director of the Max Planck Institute for Extraterrestrial Physics in Garching, Germany.

"The EU doesn't have a formal role in the funding of basic research, but that may be coming," says Genzel. "We had better be ready with a view of what should be funded."

G.B.

convincing outsiders that the discipline was worth investment, says its chair, Harold Shapiro, an economist at Princeton University in New Jersey. "They felt the field badly needed an assessment from the outside, to find out if the day of the accelerator is really over," he says. Shapiro and his colleagues concluded it wasn't, which may be of some help to US physicists when they ask for money to host the Next Linear Collider.

This imitation may give astronomers a warm glow, but it doesn't get their mirrors mounted, their balloons inflated or their spacecraft launched. The lack of progress on the 2001 committee's recommendations has become a source of anxiety, and any agreement on what to do differently in the upcoming survey depends on working out what went wrong last time.

Christopher McKee of the University of California, Berkeley, one of the co-chairs of the 2001 survey, believes that the current impasse has nothing to do with the survey process. "There have been two things that occurred in this decade that did not occur in the previous ones," he says. First, the 2003 crash of the space shuttle Columbia put a heavy financial strain on NASA. Second, President George W. Bush's redirection of the space agency towards manned exploration of the Moon and Mars drastically and unexpectedly cut its science budget. "We did not make the assumption that somehow NASA funding would increase, but we very definitely did make the assumption, at least in constant dollars, that there would be no cut in support for astrophysics," McKee says. Instead, the budget for astrophysics in 2010 is projected to be substantially below the funding level in 2000. "That is having some very major effects," McKee says. This all comes at a time when the National Science Foundation is also over-extended. Blandford is chairing a committee with the rather more challenging task of reaching agreement on which of the National Science Foundation's ground-based facilities to close down.

Others believe that the 2001 survey has to take some share of the blame itself. It's true that the budget outlook was considerably rosier during 2000, when the survey committee was finalizing its plans, says Cash. But that still doesn't explain what he describes as "a severe low-balling" of mission costs. "There was a tremendous amount of undercosting going on," agrees Chris Reynolds, an astrophysicist at the University of Maryland in College Park. "And some of it was completely apparent at the time." McKee defends the committee's choices, noting that it was wholly dependent on NASA estimates of mission costs. "We did not have any separate funding to do an independent cost analysis," he says.

Future tense

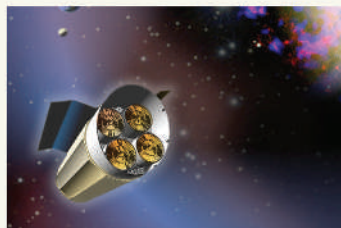
Whatever the reason for the problems, they mean that the survey that gets under way this winter will be setting a different kind of agenda, because it will have to deal with the large number of incomplete projects in the previous survey, says McKee. "I think that the survey will have to explicitly prioritize projects that have already been recommended," he says. "And it's quite possible that it will have to recommend termination of some projects."

More care must also be taken to ensure that costs are accurate, adds Strom. After the dramatic price hikes of projects in the last survey, the committee must learn to be more sceptical, he says, or it is likely to lose political credibility in Washington. "A key element of trust is a reliable cost estimate and good management tools," Strom says. "I'm not sure that kind of culture has been sufficiently inculcated by the community."

WHERE ARE THEY NOW?

The class of 2001 has had mixed fortunes.

Deferred indefinitely

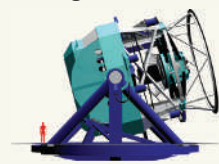


Terrestrial Planet Finder



Constellation-X Observatory

Lacking construction funds



Large-aperture Synoptic Survey Telescope

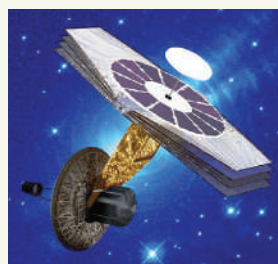


Giant Segmented Mirror Telescope

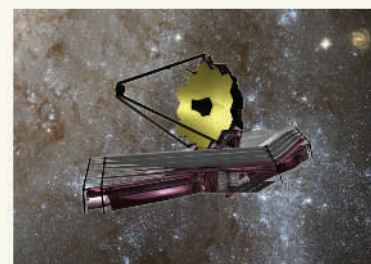


Expanded Very Large Array

Delayed launches



Single Aperture Far Infrared Observatory
Postponed to 2015–20



James Webb Space Telescope
Launching 2013

But should there be more radical changes to the way the survey is done? Some scientists think so. Reynolds believes that part of the problem is that the review depends on senior members of the community, and they may have outdated scientific viewpoints. "Those views can drive basic decisions on missions," he says. He thinks that the review should hold more public forums to get community-wide opinion and that there should be a clear and direct process for submitting project proposals. These steps would give a voice to younger researchers at the beginning of their careers.

Others criticize ranking. "I feel it's a terrible mistake to rank missions," says Cash. "If you implement it right away it's fine, but if everything sits on hold for more than three or four years, it freezes in one approach to one piece of science." Both equipment and scientific knowledge itself are changing rapidly, Cash adds. Instead of listing missions with specific costs, the survey should list the science goals of each decade, and allow missions to be chosen independently. "For example, instead of saying our number two priority is Constellation-X, we should say our number two priority is to watch blobs of plasma fall into black holes," Cash says.

But Kahn and many others continue to stand by the traditional decadal survey. "The perception within the community, which is backed by what the agencies tell us, is that the reason we've had such success is that we made hard decisions and made a list," Kahn says. "The alternative is just chaos."

Geoff Brumfiel is *Nature's* physical sciences correspondent based in Washington.



A. RAE



P. CENINI/PANOS

The ancient basilica of Emperor Justinian I still stands in Turkey's capital.

Shaken, not stirred

Hagia Sophia has stood four-square in Istanbul for more than 1,500 years. **Virginia Hughes** finds out how this venerable building has resisted the area's numerous earthquakes.

Istanbul is a city in motion. In just the past four decades, its population has exploded from 2 million to 10 million people, with migrants flowing in from the countryside for work. Most of them now live in multi-storey concrete structures, thrown up in haste and without concern for earthquake resistance.

That's a problem, because Istanbul is also literally in motion — perched as it is near the North Anatolian fault (see map). In 1999, a magnitude-7.4 earthquake killed some 18,000 people and destroyed more than 15,000 buildings in and around Izmit, a town lying 100 kilometres to the east¹. If such an earthquake were to strike the capital — and that's worryingly likely — twice as many might die, suggests a recent analysis led by Mustafa Erdik, an earthquake engineer at Boğaziçi University in Istanbul². And some 40,000 buildings could be destroyed.

Yet one of Istanbul's most famous buildings has already survived 15 centuries of earthquakes. It is a 55-metre-high, brick-and-mortar domed construction — Hagia Sophia. For decades, historians have debated how the building has withstood such seismic stress, and whether its architects planned it that way. Now, computer models and chemical analyses are providing clues to fuel these long-running debates. Were the Byzantine builders inventing new technologies, or just lucky? And when

Istanbul is hit by the next big one, will Hagia Sophia collapse — or be the only thing left standing in the rubble?

Being flexible

Hagia Sophia has endured in a land notorious for geophysical, political and religious instability. When the Muslim Ottomans invaded in 1453, they transformed it from a Christian basilica into a mosque; in 1935, the Turkish government secularized it by turning it into a museum.

The original building got its start in AD 532, at the command of Emperor Justinian I. Meaning 'holy wisdom' in Greek, Hagia Sophia was the first structure to combine the rectangular plan of traditional basilicas with the central dome of imperial buildings such as the Pantheon in Rome. Justinian spared no expense; the church cost 145,000 kg of gold (worth US\$3 billion today) and is one of the most expensive structures ever built.

And who better to build what was then the greatest church in the world than the two greatest experts of the time: Anthemius of Tralles and Isidorus of Miletus. "Anthemius was the best military engineer that Justinian had," says Ahmet Çakmak, a professor emeritus in earthquake engineering at Princeton University. "Isidorus was the director of the biggest scientific

academy in the world," he adds. "It's like you hired Oppenheimer to build your house."

Çakmak, who spent his childhood visiting Hagia Sophia, has always been fascinated by earthquakes. At 23, he left Turkey to teach at Princeton in New Jersey, and by the time Istanbul hit its population boom a decade later, he was chair of the engineering department. But it wasn't until the mid-1980s that the theoretically minded Çakmak turned his attention to practical studies of the monument from his childhood.

At the time, his engineering colleague Robert Mark was creating computerized models to show how buildings bear their loads. Mark's work explained how medieval builders' observations of mortar cracks probably led to the invention of flying buttresses³, for example. Çakmak encouraged him to make the same kind of studies of Hagia Sophia. "The technology was just beginning," Mark recalls.

Together, they made computer models that could simulate how the church moved under various conditions, such as earthquakes. They have been arguing over the results ever since.

Today's Hagia Sophia is a hodgepodge of domes, buttresses, supporting walls and minarets, added at various times in the name of religion or restoration. But, as the computer models show, the church's structural strength

comes from its original square core.

"Virtually all domed structures before this time were essentially domes on cylinders," says architectural historian Rabun Taylor of Harvard University in Cambridge, Massachusetts. In contrast, Hagia Sophia is built on the crowns of arches, which support the dome and then extend to piers that form the corners of a square. "That was new," says Taylor.

Also new were Hagia Sophia's pendentives — the concave triangular sections of brick and mortar that made smooth structural transitions between the curved tops of the four arches and the bottom of the dome.

But how did Anthemius and Isidorus plan their structure, before the discovery of calculus or Newton's force laws? Although engineers and historians disagree about the extent of their knowledge and innovation, most agree that the architects must have relied heavily on simple geometric ratios, and on the example of existing buildings such as the Pantheon.

Cracking idea

Until Mark modelled the Pantheon's dome in the late 1980s, most historians believed the windows in Hagia Sophia's dome were added solely for visual effect. There are 40 of these windows, one between each of the ribs of brick and mortar that support the 31-metre-diameter dome. Mark's research suggested that the windows were added to avoid cracking⁴. "They knew from looking at the Pantheon that that region would want to crack anyway along the axis of the windows," he explains, "so they used windows to, in a way, put the cracks in themselves."

That would have helped in seismically active Constantinople, as the city was then known. Hagia Sophia has stood, sustaining only partial damage, through more than a dozen major earthquakes. Part of this success can be attributed to its arches and pendentives, which when shaken distribute the dome's weight equally among the four underlying pillars. But



the very bricks and mortar that make up the church have also helped.

Modern earthquake-resistant buildings are constructed to be light and flexible, in order to withstand shaking. Hagia Sophia was both, far ahead of its time. Its bricks are much lighter and more porous than the bricks used elsewhere in the empire in that period. Çakmak's studies have shown that the bricks must have been baked at relatively low temperatures, less than about 750 °C, to get the right reaction between sand and lime⁵. "If it becomes higher than that," he says, "the sand becomes glassy and dense."

The original builders also used a special kind of mortar. Working with his colleagues at the National Technical University of Athens, Greece, Çakmak has found that the mortar contains a calcium-silicon compound similar to that used in today's Portland cement⁶. The high tensile strength of this mixture has allowed the church to absorb the shaking from large earthquakes, he says. In 2002, the team reported that, amazingly, after 1,500 years the calcium and silicon in the mortar could still react⁷. "The mortar cures itself," he explains. "After each shaking, there are micro-cracks, which are healed over time." (Mark, for his part, argues that the mortar's consistency may vary throughout the building, and thus cannot explain everything.)

The ratio of brick to mortar used in the build-

ing may also contribute to its strength. To build faster, the masons used extremely thick mortar joints, often thicker than the bricks themselves. The thick joints make the material "more like reinforced concrete," says Çakmak — although the technique is only possible with such a strong mortar.

Did the builders include such earthquake-resistant features intentionally? Before Hagia Sophia, says Çakmak, architects simply created very heavy buildings if they wanted them to survive earthquakes. But Anthemius was interested in the topic and even, reportedly, built his own earthquake-simulation machine. "He realized that the forces in a dynamic system are proportional to mass," argues Çakmak. "So his concept of using lighter brick and flexible mortar instead of stone makes good sense."

But Mark disagrees with his friend, coming down on the side of most architectural historians. "Ahmet thinks that they had knowledge that I don't think they had," he says.

Old reliable

Such queries aside, many wonder how Hagia Sophia will fare in the next great earthquake — the region just south of Istanbul is expected to experience two tremors of equal or greater magnitude to the Izmit earthquake in the next

few decades⁸. In 1991, a team of Turkish and US researchers fitted Hagia Sophia with several vibration sensors. From data gathered during tiny earthquakes, Çakmak created three-dimensional computer simulations that could predict

how the building might move during a large earthquake⁹. The model shows that when hit by a magnitude-7.5 tremor, the walls of Hagia Sophia will tremble and sway dramatically back and forth. The tops of its arches will feel the most stress. But the dome will remain unscathed, and the church will stand.

And if the earthquake is greater than magnitude 7.5? "If it's greater than that, there's very little we can do," Çakmak says. Indeed, if the worst earthquake predictions come true, Hagia Sophia — standing or not — will be the last thing anybody is worried about. ■

Virginia Hughes is a science writer in New York City.

**"It's like you hired Oppenheimer to build your house."
— Ahmet Çakmak**



Ancient innovation: triangular 'pendentives' join supporting arches to the building's enormous dome.

1. Parsons, T. et al. *Science* **288**, 661-665 (2000).
2. Erdik, M. & Durukal, E. *Nat. Hazards* (in the press).
3. Bork, R., Mark, R. & Murray, S. J. *Soc. Arch. Hist.* **56**, 478-493 (1997).
4. Mark, R. *Am. Sci.* **March/April** 142-150 (1987).
5. Moropoulou, A., Çakmak, A. & Polikreti, K. J. *Am. Ceram. Soc.* **85**, 366-372 (2002).
6. Moropoulou, A. et al. in *Vessex Inst. Trans. Built Environ.* **15** (eds Brebbia, C. A., Hernandez, S. & El-Sayed, M.) (WIT Press, 1995).
7. Moropoulou, A., Çakmak, A. S., Biscontin, G., Bakolas, A. & Zendri, E. *Constr. Bldg Mater.* **16**, 543-552 (2002).
8. Hubert-Ferrari, A. et al. *Nature* **404**, 269-273 (2000).
9. Çakmak, A., Moropoulou, A. & Mullen, C. L. *Soil Dynam. Earthquake Eng.* **14**, 125-133 (1995).



Fighting a reporting battle

Science journalists in the developing world face unique stumbling blocks, even as some of the biggest science stories unfold around them. **Mike Shanahan** reports.

Bennen Buma Gana, a freelance science journalist in Yaoundé, Cameroon, says he often learns about discoveries in his country from media outlets thousands of miles away. "African scientists are unknown at home," says Buma Gana, who has covered topics from tuberculosis to tropical agriculture in his three years of reporting. "We only hear of scientific research carried out in Africa from the Western media, because the studies are published first in the West."

Science journalists like Buma Gana face similar difficulties throughout the developing world, yet there is no shortage of science issues to report on. The H5N1 avian influenza virus is rampaging through southeast Asia and Africa. Malaria and HIV are entrenched. And biodiversity in many of the world's tropical regions is diminishing fast.

Sparse coverage

Reporters in the developing world face challenges that would keep many of their counterparts in the West from even trying. They are often untrained in both science and journalism, lack support and resources, and have an uneasy relationship with the scientists and officials on whom they rely for news and

comment. "If science were given a conspicuous place in the media," argues Buma Gana, "much awareness would be created and policy could change on many issues."

A spate of recent initiatives aims to bridge



"We only hear of African research from the Western media."

— Bennen Buma Gana

the gap between science and society in the developing world (see 'Spreading the word'). Buma Gana is participating in one, Science for Life (SciLife), which launched in Cameroon in April. This organization hopes to improve the quality of local science journalism by encouraging reporters and researchers to meet and work together. It plans to run seminars, training sessions and field trips to keep journalists aware of scientific developments in both Africa and the West. While it looks for an outside donor, SciLife is relying on contributions from

its members — 22 so far — to fund activities such as 'science cafés', at which the public can meet scientists and listen to talks on topics such as malaria and bird flu.

Sometimes reporters have to begin their battle in the newsroom itself, where editors are reluctant to run stories on science topics. "Science is still not on the media agenda here," says Colombian freelance journalist Lisbeth Fog. "Convincing the editor that your stories are interesting or at least easy to 'sell' is hard." When Colombian newspapers do cover science, Fog says, it is usually to report — often sensationally — on oddities such as strange animals or on controversial international issues such as genetically modified crops or cloning. "Local science rarely reaches the media," she says.

That silence is sometimes maintained even when the science involves critically important local issues. In July, the International Federation of Journalists released a study on media reporting of HIV and AIDS in some of the countries most affected by the epidemic. Coverage in South Africa was "minuscule" and in India "infrequent", the study found. In Cambodia, even in the two weeks surrounding World AIDS Day in 2005, reporting on HIV



"We depend on the rare times researchers get in touch to tell us what is newsworthy."
— Marcelo Leite

and AIDS occupied just 3% of the news.

Major media outlets in the West often have dedicated science editors, but in many developing countries there are few. It is not unknown for editors whose usual beats are sports, politics or entertainment to rework science stories out of recognition and into inaccuracy, without consulting the reporter. "There are times where you read your story the following day and just shake your head with anger at the distortion," says Ochieng' Ogodo, a freelance reporter in Nairobi, Kenya.

Secrets and mistrust

Finding news to report in the first place is challenging enough, he says, because few scientists understand the need to communicate their findings to the public. The stiff bureaucracy at most research institutions doesn't help, he adds: "It can take as long as two months to get a piece of information that should have taken just minutes to supply."

Brazilian science journalist Luisa Massarani agrees. "Trying to find good stories in Brazil or any other Latin American country sometimes feels like being in a black hole, especially when you are young and don't yet have your own contacts." Freelance science writer Marcelo Leite says this is because few Brazilian research centres have press offices that publicize their findings by issuing news releases or bringing scientists and journalists together. "We depend a lot on the rare times that researchers themselves get in touch to tell us what is newsworthy," he says.

Part of the problem is that even when scientists are keen to have their views reported on, they aren't sure how the process might play out. "Scientists fear that reporters will modify their comments in an alarmist way that could create

political or professional problems," says Egyptian geneticist and freelance science journalist Wagdy Sawahel. The result, he says, is that few scientists trust reporters.

Yet scientists are usually easier to deal with as sources than government officials, who "treat all information as a military secret", says Sawahel. "This way of thinking makes it extremely difficult to get hold of the facts about science initiatives, policies or strategic research plans."

Even in China, whose leaders regularly stress the importance of popularizing science, reporting can be difficult. Because government officials decide science policies and allocate funding mainly behind closed doors, scientists rarely feel the need to deal with the media, says Jia Hepeng, a Chinese freelancer based in Beijing. For instance, journalists were strictly forbidden from attending the World Congress of Bioethics in Beijing in August, at which policymakers and scientists were present.

When science becomes politicized, reporting becomes even tougher. Journalists who cover HIV in South Africa, which has one of the highest prevalences of the disease in the world, sometimes receive threatening phone calls from people who believe the HIV virus does not cause AIDS. The AIDS 'dissidents' have tacit — and in some cases open — approval from senior government figures including health minister Manto Tshabalala-Msimang, who has endorsed the use of vitamin pills, lemon juice, garlic and olive oil over antiretroviral drugs. An organization that favours vitamin supplements to treat HIV recently threatened to sue South African newspapers and an online news agency for critical reporting. "This has polarized newsrooms and diminished journalists' ability to report accurately," says one South African reporter, speaking anonymously.

Another source of potential controversy is the coverage of genetically modified (GM) crops, particularly in countries — such as Zambia and Zimbabwe — that have banned imports of GM grain as food aid during famines. "There are very few instances where the independent media reports on the latest developments in GM crops," says Talent Ngwande, a freelance journalist based in Zambia's capital Lusaka. A

2005 study by the UK-based Panos Institute concluded that reporting on GM crops in Brazil, India, Kenya and Zambia tended to follow the government line, whether pro- or anti-GM, and lacked a critical approach.

Long-term training

Training journalists may help. A few fortunate reporters attend international science journalism workshops each year, but those are usually one-time events. Some argue that governmental support is needed for science communication. "No university here offers science journalism courses," says Pakistani science journalist Aleem Ahmed. He, like some, has launched into science journalism on his own, creating a monthly Urdu-language magazine called Global Science that has published 100 issues over the past eight years.

To improve the lot of working journalists, the World Federation of Science Journalists is about to launch a peer-to-peer training programme. It will match 60 journalists in Africa and the Middle East with one of 16 experienced journalists, either in the same region or in the West, who will act as mentors for two years.

Many believe this could make a difference. "What makes me hopeful about this pro-



"Each mentee will hopefully receive a lot of attention."
— Nadia El-Awady

gramme is, firstly, that it is long-term," says Nadia El-Awady, an Egyptian journalist and managing science editor of IslamOnline.net. "Secondly, since each mentor is responsible for only four journalists, each mentee will hopefully receive a good deal of attention."

The programme, funded by Canada's International Development Research Center and the UK Department for International Development, is due to start this month. In November, the mentees are likely to meet with their mentors face-to-face in Nairobi while reporting on the UN Framework Convention on Climate Change.

The scheme will also twin established associations of science writers with fledgling organizations in the developing world, such as the Arab Association of Science Journalists and SciLife. Buma Gana thinks that SciLife will benefit from the scheme and, in return, bring real benefit back to society. "Talking about science from a more African point of view," he says hopefully, "could encourage our governments to invest more in scientific research." ■

Mike Shanahan, a journalist based in London, is news editor at SciDev.Net. The reporters interviewed are all freelance contributors to the site.

Spreading the word

Initiatives are arising to offer support to science journalists in the developing world.

2003: Arab science journalists set up an online discussion group to promote a scientific culture in their region. Six months later, the group forms the Arab Association of Science Journalists, now with more than 100 members.

2004: The Coalition of Journalists on Environment and Agriculture is set up in Malawi. It has organized journalistic training on environmental issues and is encouraging schools to set up science clubs.

2005: The ResearchSEA website is set up to connect research centres and

journalists in southeast Asia by publishing press releases and news. Other unrelated initiatives are launched, including the Africa Water Journalists Network, the African Federation of Science Journalists, the Costa Rican Association of Science Journalism and the Science Communicators Association of Nigeria.

M.S.

Hungary's 'reforms' are threatening basic science

SIR — Your News story “Hungary’s science academy slammed as ‘obsolete’” (*Nature* **441**, 1034–1035; 2006) rightly highlights the often-hierarchical and somewhat old-fashioned organization of the academic research scene in Hungary. A reform that would provide opportunities to successful Hungarian researchers working all over the world is indeed needed. However, by stating that “the Hungarian government... is trying to reform the country’s research system and attract more high-profile scientists”, your News story is too kind.

What the government means by ‘reform’ is budget cuts and restructuring of the increasingly limited resources provided for research. For example, the government did not hide its plans to privatize the academy-owned research infrastructure, as it was hoping to generate cash to deal with its own dire financial situation.

It is not obvious to us that selling some of its most valuable properties would help the academy to renew; on the contrary. Our concern is that a reform process driven by economic pressure would, instead, sacrifice the academy and basic research in Hungary on the altar of questionable economics. This is far from strengthening Hungarian science. We think most Hungarian scientists would agree with us, on the basis of the open letter to the prime minister, signed by some 2,500 scientists, begging the government not to cut the already lean budget allocated to basic scientific research even further (see www.petitiononline.com/otka).

Sadly, it seems that, as a central strategy of its ‘reform process’, the Hungarian government is continuing to attack basic science. It uses the populist argument that Hungary is too small and too poor to ‘waste’ taxpayers’ money on basic-research projects for which a speedy return on investment cannot be immediately identified. Hence it withdraws support from basic science and reallocates it to applied research, or research that is able to produce marketable products in a relatively short period of time.

We believe that this concept puts the basic-research network in Hungary (under the umbrella of the Academy of Sciences) into serious danger. With its short-sighted outlook, the government does not realize that its ‘reform’ activities undermine the development of a healthy innovation chain, and drown the personal creativity that has been an asset and a source of pride for Hungarian science.

Since it was founded in 1825, the primary mission of the academy has been to keep an eye on the cultural and scientific horizon in service of the nation’s long-term interests. No government

agenda formulated under economic pressure should compromise this mission.

Zsuzsanna Izsvák, Zoltán Ivics, Lajos Mátés
Max Delbrück Center for Molecular Medicine,
Robert Rösslestraße 10, D-13092 Berlin, Germany

Challenge of choosing right level of microarray detail

SIR — Your Technology Feature on microarray databases and standards, “Share and share alike” (*Nature* **442**, 1069; 2006), is incorrect to state that the National Center for Biotechnology Information’s Gene Expression Omnibus (GEO) database is not compliant with MIAME (minimum information about a microarray experiment) standards.

GEO is in fact MIAME-compliant, as it fully supports capture of all data elements defined by MIAME and encourages use of the MIAME checklist in determining what information to submit about an experiment.

Biological context is essential to interpret the underlying data in functional-genomic databases such as GEO. The challenge lies in choosing the right level of detail to require: too little and the database will not be robust enough to meet scientific demands; too much and scientists may be discouraged from submitting, or may inadvertently enter the information inaccurately. The appropriate level of biological context can vary widely between experiments, is difficult to standardize and may change over time. The level of effort a submitter is willing or able to expend may also change with shifting data-submission requirements by journals and funding agencies.

The National Center for Biotechnology Information began GEO in 2000 with a requirement for a minimal level of detail. As the field developed, we encouraged submitters to provide more specific details and modified the database design to include provisions for all MIAME data elements. The result of this incremental approach has been positive, with the growth rate and quality of GEO submissions rising rapidly over time.

Finding and maintaining the optimal balance between complexity and usefulness is challenging, as is providing appropriate tools and resources that evolve with changes in the science and social environment. GEO will continue to monitor the needs of the community and work with the Microarray Gene Expression Data Society and the European Bioinformatics Institute (which produces the ArrayExpress database), as we all seek this balance.

Ron Edgar

Gene Expression Group/National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health,
45 Center Drive, MSC6511, Bethesda,
Maryland 20892-6511, USA

Funders with money to spare could just pay more

SIR — A recent Naturejobs Prospects article (*Nature* **442**, 841; 2006) notes that Research Councils UK (RCUK) has recognized that postdoctoral salaries are insufficient, leading to problems in attracting and retaining postdoctoral staff. It would make sense for RCUK to address the problem globally, for example by increasing the baseline salaries of postdoctoral staff, or by giving principal investigators greater flexibility to set salary levels at the time of appointment.

Rather than taking such a global course, RCUK has instead offered to consider applications from principal investigators for ‘enhancements’ to individual postdoctoral salaries in strategically desirable scientific disciplines. According to the Prospects article, the organization is now complaining that it has not received enough applications for these top-up funds. RCUK should realize that this is less likely to be because principal investigators are unaware of its scheme than because of the additional administration and bureaucracy involved in providing individual justifications.

Shantenu Jha

Centre for Computational Science,
University College London, 20 Gordon Street,
London WC1H 0AJ, UK

Piltdown wasn't cricket but does the hobbit ring true?

SIR — I was surprised that you managed to discuss palaeoanthropological controversies in your Editorial “Rude palaeoanthropology” (*Nature* **442**, 957–958; 2006) without mentioning Piltdown man. The discovery of this ‘missing link’ in 1912 caused a stir, during the period between the Neanderthal and *Australopithecus africanus* disputes that you mention. Debate about it continued until it was exposed as a hoax in 1953.

Although I’m not suggesting that *Homo floresiensis* is a fake, the behaviour of the participants and those reporting on them could be drawn from that scene of palaeo-anthropological bad behaviour in Sussex. All that seems to be missing in Indonesia is a cricket bat like the one carved from bone and found at the Piltdown site, which failed to alert its finders to the fact that this was a hoax.

So let’s look forward to a season of digging to resolve the issues at hand. We could hope, perhaps, for the discovery of artefacts, tools or jewellery — rings, for example? — that might help to clarify the position of the hobbit in prehistory.

Simon L. Goodman

Friedrich Ebertstraße 102a,
64347 Griesheim, Germany

BOOKS & ARTS

The making of the modern world

The end of the Second World War heralded a revolution in the use of technology in the West.

Transforming the Twentieth Century: Technical Innovations and their Consequences

by Vaclav Smil

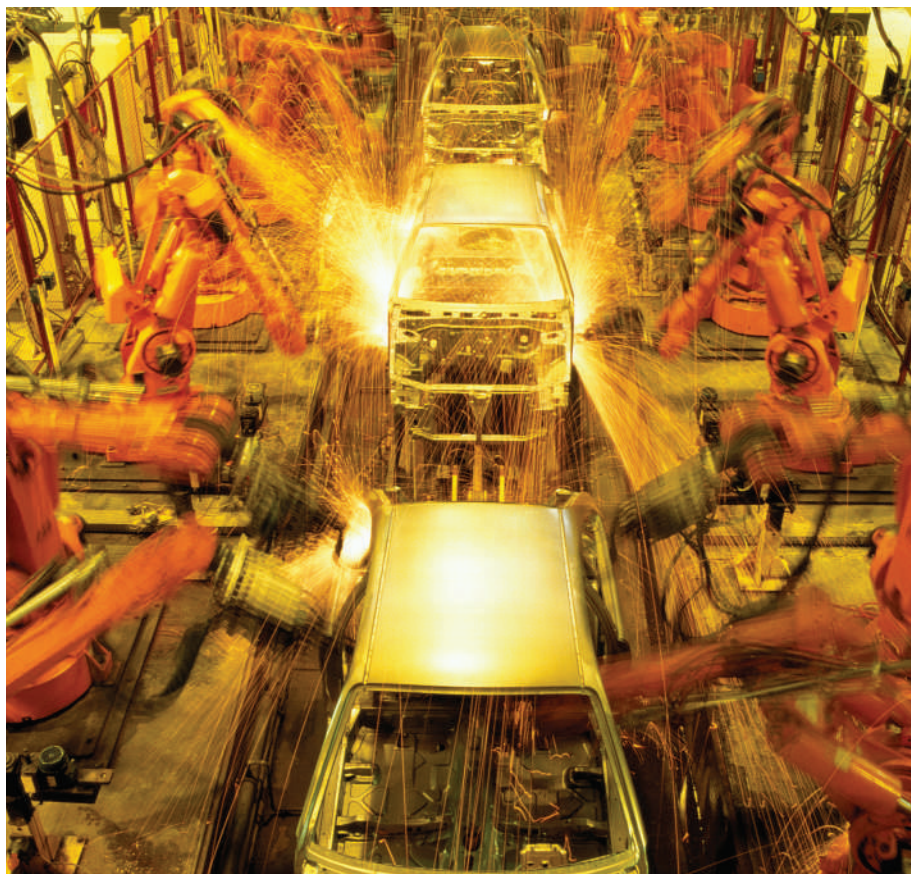
Oxford University Press: 2006. 358 pp.
\$45, £26.99

Nasir Tyabji

The Industrial Revolution was accompanied by a fundamental change in the evolution of technology in Western society. In technological terms it is the historical dividing line between an era of heuristic advances in production systems and a time of focused application of the scientific approach to increasing productivity. But historians also recognize the importance of the chemical revolution of the late nineteenth century. This saw the introduction of continuous industrial processing, signalling a new relationship between human labour and the materials on which they worked, although the manufacturing process was still dominated by batch production. The next major change, just after the Second World War, was the scientific and technological revolution, which potentially heralded the end of the millennia-old systems of the mechanical processing of materials, replacing them with biological, chemical and molecular-level production processes.

J. D. Bernal was the key figure behind studies of technology's role in history, influenced heavily by the decisive interventions of the Soviet delegation to the 1931 Congress of the History of Science in London. In particular, Boris Hessen's paper, by demonstrating how even Newton's most abstract concerns in *Principia* were profoundly influenced by the political economy of the time, reshaped the way in which the history of science would be viewed. Bernal's ideas were subsequently developed in the Soviet Union and the Eastern bloc, where some very interesting work was done in the 1970s and 80s. However, no significant work on this has emerged from there since the collapse of the Soviet Union.

The imprint of that Eastern European tradition seems to remain with Vaclav Smil, even though he moved from Czechoslovakia to North America in 1969. His book *Creating the Twentieth Century* (Cambridge University Press, 2005) described the impact of post-1870s technological developments on society, and his new book, *Transforming the Twentieth*



S. ROWELL/GETTY

The automated production line symbolizes the manufacturing advances of the late twentieth century.

Century, carries on where the first left off. Between them they cover both the continuous-processing, and the scientific and technological revolutions.

In *Transforming the Twentieth Century*, Smil describes in detail the changes brought about by two distinct processes. The first is the development of innovations originally introduced between 1867 and 1914, whose full impact only became apparent after the Second World War. He then describes the effects of the post-war introduction of microelectronics, new materials and different methods of processing. In separate chapters he presents case studies of changes in energy conversion, in the development and uses of new materials, in the methods of production and, finally, in means of transportation, communication and information processing. He includes real technological change (which involves changes in the knowledge systems of material production), along

with advances in technological artefacts (such as aircraft and means of railway locomotion) and technological systems (the Internet). The book, in effect, examines the making of modern society through technological change. This approach, which was pioneered by Siegfried Giedion some 60 years ago in his book *Mechanization Takes Command*, has the advantage of making the overwhelming impact of technological change more easily apparent by considering its effects on everyday life. (Giedion, incidentally, is absent from the bibliography.)

In developing his reasons for writing the book, Smil raises several interesting points. He notes that the great increase in the fuel efficiency of North American cars after 1973 came not from market-based competition, but from government legislation that required greater economy in the use of fuels. Equally important is his methodological observation that innovations are often the result of

“successful transformative engineering: a system assembled from proven ingredients whose synergies change a mundane experience to such an extent that it becomes not just new but highly desirable”.

These comments, and others scattered throughout the introductory chapter of the book, raise expectations of broader insights to be drawn from the detailed case studies presented later. However, subsequent chapters give an unmistakable sense that Smil has lost control of the process by which his argument might develop, among the details of the case

at hand. As a result, the case studies remain in the form of encyclopaedic entries, rather than instances by which the nuances of a more general and complex argument might have developed. To be fair to Smil, he notes in the preface that he has no intention of proposing a great thesis. But the reader cannot help feeling that the enormous effort expended in collecting information throughout the book deserves a rather greater degree of conceptual closure. ■ Nasir Tyabji is director of the Centre for Jawaharlal Nehru Studies, Jamia Millia Islamia, New Delhi 110 025, India.

Science uncut

Sex, Drugs and DNA: Science's Taboos Confronted

by Michael Stebbins

Macmillan Science: 2006. 360 pp.

\$15.72, £16.99

Lee M. Silver

I have fond memories of my middle years as a graduate student working in the Harvard Bio Labs during the mid-1970s. Behind the life-size bronze rhinos that guarded the building, lights burned all night long as my fellow students and I pipetted solutions, ran gels and played with expensive equipment, all to the beat of blasting rock music. Like kids in a sweetshop, we would ‘do science’ to our hearts’ content until we dropped from exhaustion. The next day, we’d wake at some point, throw on some tatty clothes, and think up new experiments. Our chief diversion was smoky weekend parties where a few hip professors would join us, often with an eye for female grad students. On one occasion, a lab-mate synthesized mescaline — following a recipe from *Nature* — and we had a grand time tripping through Harvard Square and along the banks of the Charles River. The only laws that mattered were the laws of nature, and as far as we were concerned, even those were ripe for attack.

Why was the US government footing the bill for our anarchic endeavours? As long as money flowed into experimental reagents, lab supplies and our measly stipends, we didn’t have a clue and we didn’t care. With no classes or exams, no children to feed, no possessions of any worth, and not much thought for the future, we could occupy ourselves completely with the subjects covered by *Sex, Drugs and DNA*, Michael Stebbins’ book-length rant about the irreverent lives and world-views of molecular biologists.

Stebbins tells us at the start that he intends to “address the litany of bullshit and lies” that other scientists are afraid to talk about in public. What are some of the hushed-up truths? Genes play a role in behaviour; men and women have anatomically distinct brains; professors can

sleep with grad students, as long as they’re discreet about it; students entering grad school have no idea what they’re in for, and how small their chances are of getting a coveted academic position; there’s no good time for an aspiring woman scientist to have a baby; scientists can be selfish and petty; and they can curse with as much abandon as a drunken football fan. Many colleagues will find nothing to quarrel about in these accounts. But how many others will be interested in the anthropology of the cultish molecular-biology tribe is not clear.

Stebbins’ ruminations run far beyond work practices, dancing across nearly every major intersection of biological science with society. Some of his observations are right on the mark, such as an in-depth look at the corrupt politics and fraudulent marketing of ‘dietary supplements’. Sometimes, however, particularly when he glibly dismisses objections voiced by the ‘religious right’, Stebbins is guilty, in reverse, of the sin he pins on his ‘ignorant’ opponents. He writes, for example: “there is nothing in the

theory of evolution that really threatens the Christian faith”; opposition to contraception is based on “ridiculous hypocrisy and religion-based stupidity”; and “none of the arguments” made against embryonic stem-cell research “made much sense”. Comments such as these fail to provide much insight into these raging controversies.

Scientists should be more suspicious when Stebbins readily accepts the simplistic conventional wisdom spouted by people on the political left about complex environmental issues. He demonizes DDT, for example, as an agent of human birth defects, without providing any quantitative information and without mentioning the role it played in eradicating malaria from US and European shores. Indeed, DDT could save the lives of millions of African children, if used wisely, without any significant negative impact on the environment or health.

Stebbins also reflects the naivety I expressed as a graduate student regarding the larger purpose of government funding of university research. And like many academic scientists, he hasn’t delved into economics sufficiently to understand that market forces and intellectual-property rights provide the greatest incentive for the rapid exploitation of basic science, with respect to the development of pharmaceuticals and other biotech products.

Nevertheless, if you want a taste of the off-the-cuff, vulgarity-tinged musings you’re likely to hear after hours in a pub from a bunch of sometimes-uninformed molecular biologists, then, by all means, read this book. ■

Lee M. Silver is at the Woodrow Wilson School of Public and International Affairs, Princeton University, Princeton, New Jersey 08544, USA. He is the author of *Challenging Nature: The Clash of Science and Spirituality at the New Frontiers of Life* (Ecco Press).



Science and society: students have often engaged with political issues by mounting protests to wars.

BETTMANN/CORBIS

Border crossings

Biodiversity hotspots don't often fit neatly within political borders, and most span at least two or three countries. Establishing transboundary conservation areas is a complex and difficult process, but one that can bring socioeconomic benefits to a region as well as conserving its biodiversity.

Transboundary Conservation: A New Vision for Protected Areas (Conservation International/Agrupacion Sierra Madre, \$50) by Russell A. Mittermeier *et al.* introduces the history of this type of conservation initiative. It surveys examples from every continent, including the first, Waterton-Glacier International Peace Park between Canada and the United States, established in 1932, and the largest, the Great Limpopo Transfrontier Park. Antarctica, the last global commons (pictured here), is also included in this book, which uses the power of photography to help propel the conservation agenda.



P. OXFORD/MINDEN PICTURES

The moral element

Against Bioethics

by Jonathan Baron

MIT Press: 2006. 248 pp. \$29, £18.95

Prashanth Ak

How should public-policy issues that involve an ethical component be decided? Should we rely on moral intuition? Can we apply some standard rules? Advances in medical and biological research mean that we continue to face these age-old questions in new situations. How should we legislate genetic manipulations or triage healthcare resources? In *Against Bioethics*, Jonathan Baron argues that current bioethical practices are predominantly based on “tradition and intuitive judgments”, and he proposes instead a systematic approach to bioethical analysis and decision-making.

Most readers of *Nature*, trained as we are in the scientific process, will have no difficulty in agreeing with the need for a clear, systematic analysis and evaluation of possible outcomes, such as that advocated by *Against Bioethics*. But how do we proceed? What framework do we use? In an admirably concise, lucidly written exposition, Baron proposes that we use decision analysis, with utilitarianism as the moral framework. He addresses specific problems such as drug research, death and end-of-life issues (including advance directives, euthanasia and organ donation), informed consent and the practices of institutional review boards. The book delivers a praiseworthy analysis of problems in these areas with a rigorous application of clear, consistent methods.

However, there is a major impediment to the wide acceptance of the methods he proposes: the utilitarian framework is controversial, and various objections have been raised against such frameworks before.

Moral theories fall into roughly two categories. The first kind bases the rightness or

wrongness of an act on the consequences (for example, cheating is wrong because of the bad consequences it produces). The second category claims that rightness or wrongness is inherent in the nature of the act itself (cheating is morally wrong, even when it produces good consequences). An example of the first category, utilitarianism, simply stated, posits that a moral act is one that maximizes utility, or ‘good’ consequences. It has permeated Western intellectual life as a philosophy over the past two centuries, especially in law, politics and economics. But its influence has been counterpointed by deontological ideas, such as morality independent of consequence, and inalienable fundamental rights. Indeed, much of current thinking on morality and ethics in the Western world has been an unsettled balance between such deontological ideas and utilitarianism. This uneasy truce, in various forms, spans the history of Western civilization — ever since the young Socrates, using utilitarian ideas, vigorously rejected the Sophists’ claims of common morality. The debate continues.

Against Bioethics is not intended as a defence of utilitarianism *per se*, and is unlikely to sway its opponents. Acting to maximize ‘good’ consequences seems sensible, but whether the whole of normative ethics can be analysed in these terms is far from settled. If one is part of a minority that frequently has to make sacrifices for the good of the majority, it can get tiresome in the long run. ‘Good’, in any case, is difficult to define, let alone measure. Partly because of this difficulty, various modified and complicated formulations of utilitarianism exist today, with different definitions of ‘good’: as pleasure (by Jeremy Bentham); happiness (John Stuart Mill); ideals, such as freedom, justice and beauty (George Moore); preferences, or satisfaction by things of intrinsic value

(Richard Hare). Regardless of whichever definition of ‘good’ is acceptable (and for the most part, *Against Bioethics* seems to favour ‘preferences’), measuring ‘good’ is difficult, especially over time and across a pluralistic society. It is sobering to note that Henry Sidgwick’s 1874 volume *The Methods of Ethics*, arguably the most detailed and subtle exposition of utilitarianism available, concludes: “it would seem necessary to abandon the idea of rationalizing [morality] completely.”

A significant feature of *Against Bioethics* is the application of decision analysis to bioethical problems. An offshoot of statistical decision theory, decision analysis enables an optimal choice from a set of alternatives in the face of uncertainty (allowing the use of probabilistic criteria where possible). It is similar to utilitarianism in that decisions are made on the criterion of maximization (of utility, for example). Both claim that, in principle, all relevant considerations can be reduced to a utility function, which allows for comparisons. However, decision analysis is ‘morally blind’ as it does not cater to any particular moral framework — it can easily incorporate utilitarian or non-utilitarian concerns. Decision analysis as a procedure for systematically analysing bioethical problems would perhaps be more acceptable if it were not used within a utilitarian framework as *Against Bioethics* proposes.

There is a need to address bioethical issues, and in *Against Bioethics* Baron at least gives us pause for thought. Its starting point, that much is lacking in current bioethical practices, is certainly worth considering. All too often in bioethical decisions, competing principles are intuitively balanced and applied ad hoc. Development of a thorough, coherent theory to guide and systematize practices and policies can only help. Initiatives such as this book are especially welcome, and will hopefully serve to instigate debate and discussion.

Prashanth Ak is at the Genome Center, University of California, Davis, California 95616-8816, USA.

Intellect and intuition

An Idealist View of Life

by Sarvepalli Radhakrishnan

George Allen and Unwin: 1932

Gautam R. Desiraju

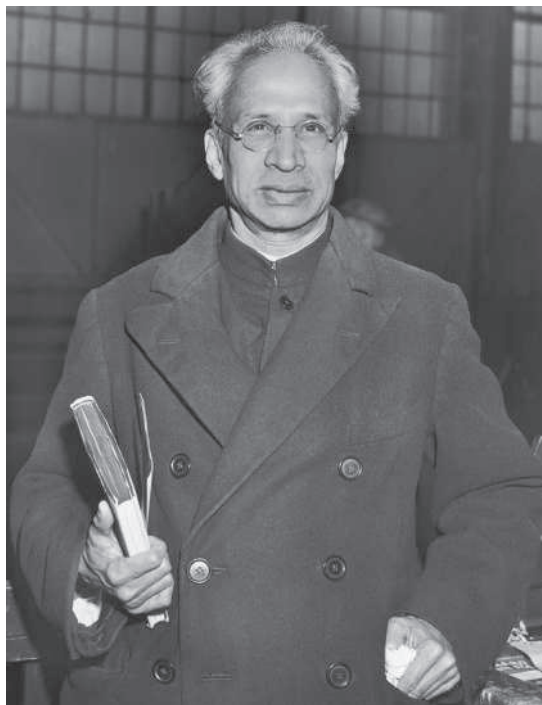
Sarvepalli Radhakrishnan was in his time the most renowned interpreter of Indian philosophy to an international audience. In *An Idealist View of Life*, based on the Hibbert lectures he delivered at the universities of London and Manchester in 1929–30, he presents his thesis on the nature and meaning of life.

The book weaves a spiritual approach through its metaphysical argument, also adopting a scientific attitude, to produce a bold view of creative thinking. Radhakrishnan exposes the inadequacy of existing religions and contrasts them with science, which “demands induction from facts and not deduction from dogmas”. Speaking amid the gathering storm of the impending war in Europe, he is disappointed by the moral inadequacy and destructive political consequences of established faiths, and states that “nothing is as hostile to religions as other religions”. If religion is ineffective, the new gods of atheism, agnosticism and authoritarianism are equally without worth, and he describes the modish sceptic as someone for whom nothing is serious and who “drifts along confusedly, hoping for the best, expecting little and believing in nothing very much”. This is not an inaccurate description of many scientists today, for whom the deeper meaning of science remains elusive in an ever-changing yet constant world.

According to Radhakrishnan, there are two forms of knowledge: intellectual and intuitive. Intellectual knowledge lies at the core of Western systems of philosophy and is based on critical analysis and logic. If all knowledge were purely intellectual, reason alone would suffice, eventually leading to hard-core reductionism. However, Eastern systems of philosophy, particularly Hinduism, believe in a higher form of knowledge built on intuition. Intuition is essentially synthesis, which in turn follows naturally from Hindu ideas of monism. According to advaitic (non-dualistic) doctrine, one who knows the Absolute becomes the Absolute. Accordingly, ignorance of this knowledge is the root of all trouble.

It is understandable thus far. But

Radhakrishnan then claims that the true test of knowledge is certainty, which is a characteristic of intuitive thinking, rather than communicability, which characterizes intellectual knowledge. He then argues that intuition does not oppose intellect but rather lies beyond it. In other words, intuitive knowledge, although it does not contradict the necessity of reason, questions the sufficiency of reason. Intuition is not illogical but super-logical. In summary, both intellect



Sarvepalli Radhakrishnan combined spirituality and science.

and intuition must be synergized in the quest for the nature of ultimate reality.

Addressing scientific issues, Radhakrishnan argues that “great scientific discoveries are due to the inventive genius of creative thinkers”, intuitive thinking being as relevant to science as it is to the arts. Consider, for example, Faraday’s discoveries, Mendeleev’s periodic table, Kekulé’s postulation of the cyclic structure for benzene, or the way in which Crick and Watson unravelled the double-helix structure of DNA.

Several hypotheses in Radhakrishnan’s book are worthy of close attention from scientists. First, he says that great intuition arises from a bedrock of rationality. Next, when the big discovery is made, there is enough room for the readjustment and reinterpretation of partial and incomplete concepts and data that preceded the discovery. This readjustment is so simple,

says Radhakrishnan, that when full scientific insight is obtained it escapes notice, allowing one to imagine that the process of discovery arose entirely from intellectual thinking. Finally, instinctive thinking is not easily communicable — so when the discovery is discussed, it appears to have arisen from rational thought. The lines between discovery and proof are now blurred, resulting in an erroneous simplification of the deeper movements of thought.

There is much to commend this approach to scientists today, given the increasing complexity inherent in contemporary scientific problems. The coming century will see an intensive study of emergent phenomena such as the prediction of crystal structures, protein folding, fractals and monsoon forecasting. The answers to these difficult questions may not be found in a single model of complexity, such as cellular automata. Eventually, we would even like to examine the differences between life and non-life in, say, chemical terms. Subjects such as physics and chemistry are already redefining their goals to study more complex biological phenomena, with modern chemical biology being a pertinent example of this trend.

As the natural sciences move towards the social sciences and less precise forms of knowledge, there is a real need for inspired intuition, not as a substitute for thought but as a challenge to intelligence. An

intuitive and holistic grasp of the situation is required. What we need is not faster computers, but faster minds.

The final section of the book seamlessly synthesizes the role of intuition in the cognitive, aesthetic and ethical endeavours of humankind. Recognizing these efforts as varied facets of human expression, Radhakrishnan opines that only religion based on spiritual values can completely encapsulate the human spirit. At a time when religious fundamentalism threatens the stability of the world, there is an urgent need for intuitive and inquiring scientists to contemplate the eternity between the atom and the Absolute. As Radhakrishnan says: “Real heroes are religious in a true sense in that they have broken down the barriers between the individual and the universal.”

Gautam R. Desiraju is in the School of Chemistry, University of Hyderabad, Hyderabad 500 046, India.

NEWS & VIEWS



J. B. SHURIN

A North American bog, home to pitcher-plant communities.

ECOLOGY

A pitcher of things to come

Jonathan B. Shurin

Big conclusions can be drawn from the tiny ecosystems that flourish in carnivorous pitcher plants. Manipulating habitat size and predator abundance reveals which is more important to ecosystem dynamics.

The creation by humans of novel combinations of species and environmental conditions is rapidly driving Earth's ecosystems into uncharted territories. Information about ecological interactions can be gleaned only with great investment of time, effort and money. Thus, creative approaches are required to extract the greatest insight into these changes from the fewest data.

Writing in *PLoS Biology*¹, Gotelli and Ellison wrestle with this problem, and show how simple experiments can illuminate a network of community interactions. The immediate subject of their interest is pitcher-plant communities. These diverse groups of insects and microorganisms, living in tiny pools of water (phytotelmata) in the leaves of carnivorous pitcher plants, are found in bogs across North America (pictured). The authors subjected these communities to experimental tests, and used novel statistical approaches to disentangle the ensuing chains of indirect responses.

Reconstructing the response of organisms to past change is a sufficiently challenging task to occupy an entire discipline, palaeoecology. Predicting the effects of future changes is an endeavour so fraught with uncertainty that it demands a critical evaluation of the complexity required of ecological models. Biological

communities display an intricacy that defies generalization, with diverse populations linked by interactions that may be cryptic, or exhibit unexpected or context-dependent behaviour. The number of potential interactions between species increases as the square of their number², so putting the pieces together through exhaustive experimentation is prohibitively time-consuming.

In addition, indirect interactions among species are crucial for predicting a community's response to environmental changes, and can sometimes overwhelm the direct effects of perturbations on individual species. For instance, mid-range ultraviolet light (UV-B) inflicts damage on algae in streams. Removing these harmful wavelengths might therefore be expected to enhance primary production. But it turns out that the insects that graze on the algae are even more susceptible to UV-B sunburn, and so sheltering streambeds from these rays enhances the insects' survival, resulting in reduced algal populations³.

It has been argued⁴ that such chains of indirect interactions are so minutely sensitive to environmental conditions that communities starting from very similar initial conditions may follow disparate paths, ending up with wildly different outcomes. The dynamics

of ecological communities that are subjected to a changing environment might therefore be fundamentally unpredictable: it could be that future conditions can never be anticipated on the basis of models constructed in the present.

Gotelli and Ellison¹ show how an approach of intermediate complexity can extract the most important interactions in a community and so predict its dynamics. They manipulated the volume of their pitcher-plant habitat and the presence of dipteran larvae (the most important predators in pitcher plants), and measured the response to these changes of the aquatic mites, rotifers, protozoans and bacteria living there.

Rather than simply testing the direct effects of their manipulations, the authors compared their data with different proposed configurations of direct and indirect interactions among the target species. They used so-called path modelling to propose different routes by which the impacts of their manipulations were transmitted among competitors, predators and prey. The model that showed the best agreement with the data identified the most important interactions among species.

They found that a food-web model that included only the effects of predator density

and indirect interactions always offered the best agreement with the data, and so the best predictive power. Surprisingly, including habitat volume did not improve the fit of the model to the data — even though this volume was varied over an order of magnitude, and had significant effects in analyses where it was the only variable. The implication is that the effects of habitat loss on the community can be explained entirely as the indirect consequence of its impact on the top predator. (The top predator is most likely to be affected by habitat loss, as it requires the largest volume to persist.)

Gotelli and Ellison's experiment¹ offers important lessons. First, given their small numbers, predators often exert a disproportionately strong influence over the communities in which they live, but their rarity makes them highly vulnerable to habitat loss. This finding agrees well with the cascade of effects

observed in larger ecosystems, for example when top predators such as jaguars were lost from tropical forests on Venezuelan islands following habitat fragmentation⁵. Many plants that could themselves easily cope with the loss of habitat felt the impact of the changes from above, as the extirpation of carnivores led to increases in the herbivores that were the carnivores' prey, but the plants' predators. Bromeliad phytotelmata in Costa Rica tell a similar story. The water held inside the leaves of these plants provides a microenvironment similar to that of pitcher plants. Here, increased structural complexity of the habitat (a larger number of leaves) affected communities largely by reducing the foraging efficiency of predatory damselfly larvae⁶.

Second, Gotelli and Ellison show how habitat and predator losses percolate through just a few strong community links, such as the interaction between bacteria and their single-celled

predators. Communities can be dominated by many weak and a few strong interactions⁷; identifying these few strong interactions therefore points to a simpler path to predicting community dynamics. It offers some hope to those who despair of understanding ecological communities well enough to predict their behaviour in a rapidly changing global environment. ■

Jonathan B. Shurin is in the Department of Zoology, University of British Columbia, 2370-6270 University Boulevard, Vancouver, British Columbia V6T 1Z4, Canada.
e-mail: shurin@zoology.ubc.ca

1. Gotelli, N. J. & Ellison, A. M. *PLoS Biol.* **4**, e324 (2006).
2. Martinez, N. D. *Am. Nat.* **139**, 1208–1218 (1992).
3. Bothwell, M. L., Sherbot, D. M. J. & Pollock, C. M. *Science* **265**, 97–100 (1994).
4. Yodzis, P. *Ecology* **69**, 508–515 (1988).
5. Terborgh, J. et al. *Science* **294**, 1923–1926 (2001).
6. Srivastava, D. S. *Oecologia* **149**, 493–504 (2006).
7. Paine, R. T. *Nature* **355**, 73–75 (1992).

CHEMISTRY

Hydrogen at the flick of a switch

Masanori Takimoto and Zhaomin Hou

Before hydrogen can be used as a transportation fuel, a safe storage system for the gas must be found. Metal clusters that release hydrogen in response to an electric current may be a step in the right direction.

With mounting concern over the environmental impact of oil as a fuel, hydrogen increasingly looks like a useful alternative. In principle, hydrogen can be generated in a clean way from water by using sunlight in combination with solar cells. Moreover, it is non-polluting, and forms an environmentally benign by-product — water — on combustion. Hydrogen is thought to be an ideal fuel for vehicles, but its widespread use is limited by the lack of a safe, efficient system for on-board storage. The density and the condensation temperature of hydrogen are very low (–252 °C at 1 atmosphere), which makes it difficult to use conventional storage systems such as high-pressure gas containers or cryogenic liquid-gas containers. Therefore, the development of safe and convenient methods for hydrogen storage is an active research area¹. In *Angewandte Chemie*, Weller and co-workers (Brayshaw et al.²) report that hydrogen may be stored and released using molecular clusters, in a process that is easily controlled by a simple chemical reaction or by an electric current.

One of the most successful and extensively studied methods for hydrogen storage is to keep the gas in a 'hydride' form^{3,4}. In this approach, an alloy absorbs and holds a large amount of hydrogen by chemically bonding with the gas to form metal hydride compounds⁴. But although a hydrogen-storage alloy can absorb and release hydrogen without compromising its own structure, heating is required to promote

the release, as this process takes up energy. The alloys studied so far usually require a temperature of about 300 °C to provide hydrogen at 1 atmosphere pressure.

In contrast, the method now reported by Weller and colleagues² enables hydrogen storage and controlled release without a large input of energy. Their system is based on an organometallic compound that contains a core of six rhodium atoms⁵, as part of a complex that also includes 12 hydrogen atoms (Fig. 1). This cluster absorbs two molecules of hydrogen (H₂) to produce a compound holding 16 hydrogen atoms. The absorption process takes 10 minutes at room temperature under 1 atmosphere pressure of hydrogen, and is almost instantaneous under 4 atmospheres of hydrogen^{6,7}. The absorbed hydrogen molecules are retained at room temperature for weeks without any external hydrogen pressure (under an inert atmosphere of argon), but can be removed under vacuum to quantitatively regenerate the 12-hydrogen cluster, although this takes a long time (several days) compared with the uptake process.

Remarkably, Weller and colleagues² have found that hydrogen release from the 16-hydrogen cluster can be dramatically accelerated simply by changing the cluster's oxidation state. Adding a reducing agent to a solution of the 16-hydrogen compound releases one molecule of hydrogen from each cluster, so yielding a product containing

14 hydrogen atoms (Fig. 1). The original 12-hydrogen cluster is then easily regenerated by treating the 14-hydrogen cluster with an oxidizing agent, liberating another hydrogen molecule and completing the hydrogen uptake–release cycle.

Another crucial finding by Weller and colleagues² is that a rapid hydrogen uptake–release cycle can also be accomplished electrochemically — that is, the reduction and oxidation steps are achieved directly by electron transfers at electrodes. Adding one electron to the 16-hydrogen cluster liberates a hydrogen molecule, giving the 14-hydrogen cluster described above. This rapidly loses another hydrogen molecule to yield a 12-hydrogen cluster, which differs from the original starting material by having only one positive charge — the original 12-hydrogen cluster has two positive charges. The electrochemical hydrogen-release process occurs in a matter of milliseconds on a glassy carbon electrode at ambient temperature and pressure. The product of this process is easily converted back to the original 12-hydrogen cluster by electrochemical oxidation (electron removal at an electrode; Fig. 1). The overall electrochemical process of hydrogen uptake and release can be repeated at will.

With the aid of theoretical calculations, the authors² inspected the electronic structures of the starting 12-hydrogen cluster and of the 16-hydrogen cluster in this hydrogen-storage system. They found that the energy level of

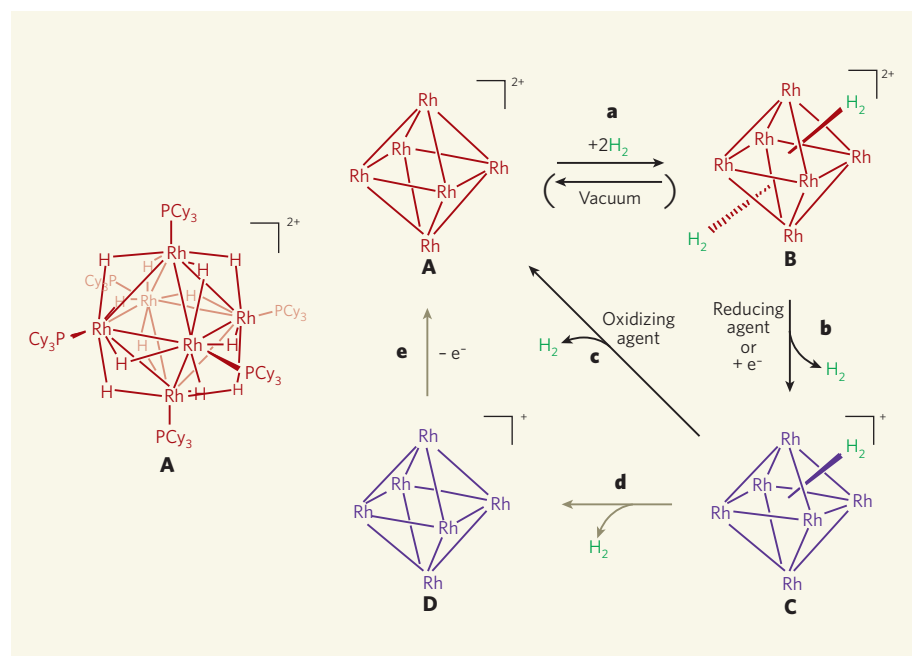


Figure 1 | Hydrogen uptake–release cycle in molecular clusters. Structure A is the hydrogen-storage material developed by Weller and colleagues²; Rh represents rhodium atoms, and PCy₃ are bulky molecules bound to the metals. Clusters in the same colour are in the same oxidation state (also indicated by the number of positive charges on the clusters). The structures are simplified in the depicted hydrogen uptake–release cycle. **a**, The 12-hydrogen-atom cluster (A) takes up two hydrogen molecules to form a 16-hydrogen-atom cluster (B). These hydrogen molecules may be removed under vacuum. **b**, The release of one molecule of hydrogen from B is promoted by a reducing agent or by the transfer of an electron (e[−]) from an electrode, to give the 14-hydrogen-atom cluster (C). **c**, Chemical oxidation of C promotes the release of one molecule of hydrogen, regenerating the starting material A. **d**, Under electrochemical reduction conditions, the release of one molecule of hydrogen from C occurs spontaneously, yielding a 12-hydrogen-atom cluster (D). This cluster is not at the same oxidation state as A. **e**, Electrochemical oxidation of D regenerates the starting material A.

the lowest-energy molecular orbital that lacks electrons (known as the lowest unoccupied molecular orbital, or LUMO) in the starting material is only slightly higher in energy than that of the highest electron-filled orbital (the highest occupied molecular orbital, or HOMO). This is why the LUMO readily accepts electrons donated from hydrogen molecules. In contrast, the 16-hydrogen cluster has a large HOMO–LUMO gap; the addition of an electron into the LUMO destabilizes the molecule, and induces the release of a hydrogen molecule.

The hydrogen-storage capacity of this rhodium system, expressed as the ratio of the

mass of releasable hydrogen to that of the storage system, is only 0.1%. This is clearly not sufficient for practical applications — the US Department of Energy wants hydrogen-storage systems to have a capacity of 6% weight-for-weight by 2010. Improved materials must be developed with a greater number of usable hydrogen molecules, bound to clusters of metals with an overall lower molecular mass. Nevertheless, this work² provides a well-defined molecular model and a worthwhile strategy for the development of hydrogen-storage materials with high efficiency and convenience.

Masanori Takimoto and Zhaomin Hou are at RIKEN, the Institute of Physical and Chemical Research, Hirosawa 2-1, Wako, Saitama 351-0198, Japan. e-mails: mtakimoto@riken.jp; ouz@riken.jp

- Schlapbach, L. & Züttel, A. *Nature* **414**, 353–358 (2001).
- Brayshaw, S. K. et al. *Angew. Chem. Int. Edn* **45**, 6005–6008 (2006).
- Schüth, F., Bogdanović, B. & Felderhoff, M. *Chem. Commun.* 2249–2258 (2004).
- Sandrock, G. *J. Alloys Compounds* **293–295**, 877–888 (1999).
- Ingleson, M. J. et al. *J. Am. Chem. Soc.* **126**, 4784–4785 (2004).
- Brayshaw, S. K. et al. *Angew. Chem. Int. Edn* **44**, 6875–6878 (2005).
- Brayshaw, S. K. et al. *J. Am. Chem. Soc.* **128**, 6247–6263 (2006).

EVOLUTION

Different paths to the same end

Antonis Rokas

Genetic dissection of a yeast gene-regulatory pathway shows that the logical output of such a pathway can remain the same even though the molecular mechanisms underlying the output have diverged remarkably.

From penguins to mushrooms and baobabs, the world around us harbours a bewildering diversity of life forms. Much of the evolution of this diversity is due to changes in the underlying genetic regulatory architecture¹. But what happens to such architecture when organisms that diverged long ago retain the same traits (or ‘phenotypes’)? Can this regulatory architecture diverge while the overlying phenotypes remain similar? On page 415 of this issue, Tsong et al.² examine the gene-regulatory circuit that governs mating type in several yeast species, and they identify a remarkable example of divergence at the genotypic level (the DNA sequence) despite conservation at the phenotypic level.

The yeast species *Saccharomyces cerevisiae* and *Candida albicans* are seemingly very different: *S. cerevisiae* is the cornerstone of the baking and brewing industries, whereas *C. albicans* is the most commonly encountered fungal pathogen in humans. Both yeasts have one thing in common, however — sex. Although yeasts do not have different sexes *per se*, both species have two molecularly distinct mating types. Mating type in yeasts is controlled by the MAT genetic region (locus), which exists in two versions, MAT α and MAT a . A cell’s mating type is determined by which version it expresses. Thus, cells expressing the a protein become a cells by expressing a -specific

genes (asgs), and are specialized for mating with yeasts of the opposite mating type — α . Likewise, α -expressing cells become α cells, express α -specific genes (asgs), and can mate only with yeasts of the a type.

Underlying this apparent similarity in mating phenotype in the two species (a -expressing cells become a cells, and α -expressing cells become α cells), the detailed genetic mechanisms by which mating-type identity is achieved seem to diverge considerably (Fig. 1, overleaf). In the a cells of *C. albicans* the asgs are only expressed once they have been activated by the $a2$ protein, whereas in *S. cerevisiae* the MAT $a2$ gene (encoding the $a2$ protein) is

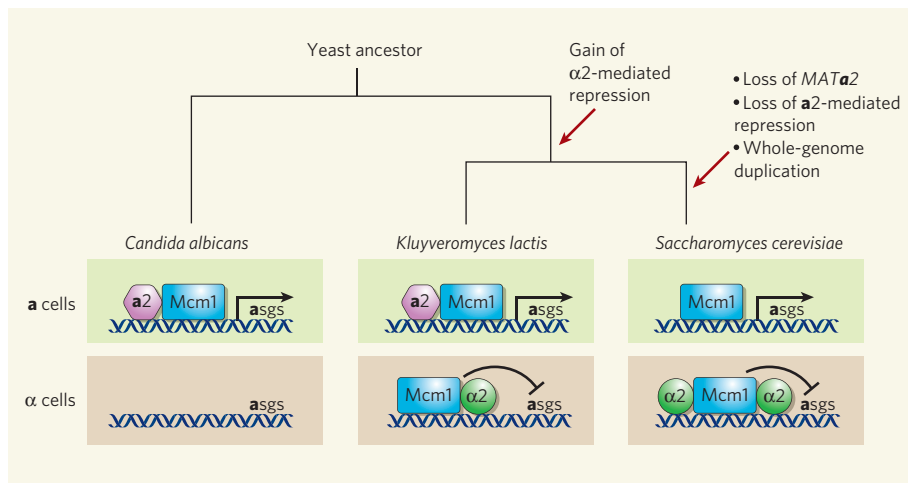


Figure 1 | The divergent regulatory architectures associated with yeast mating type. Tsong *et al.*² find that in three distantly related yeasts the regulatory architectures are remarkably divergent. This is despite the conservation of the regulatory logic: specifically, in all three yeasts, *a*-specific genes (*asgs*) are turned on in *a* cells and turned off in α cells. The Mcm1 protein is equally expressed in both cell types and is required for both *asg* and α *sg* regulation, sometimes in concert with the *a2* and α 2 proteins. Major transitions in the regulatory architecture are indicated by red arrows.

absent and the *asgs* are expressed by default in its *a* cells. In the case of α cells, the α 1 protein activates *asgs* in both yeasts, but in *S. cerevisiae* α 2 is also required to repress the *asgs* (otherwise they are always 'on').

As the regulatory architecture in *C. albicans* also occurs in an evolutionarily diverse set of other yeasts, it is most probably closer to the ancestral one. If so, two key modifications must have occurred in the regulatory architecture of a direct ancestor of *S. cerevisiae*, namely the loss of the *MATa2* gene and the gain of *asg* repression by the α 2 protein.

To piece together this evolutionary path, Tsong *et al.*² examined how *asgs* are regulated in each species. Having identified the set of *asgs* in *C. albicans*, they then surveyed the regions in front of all *asgs* to identify putative binding sites for gene-regulatory factors. In *S. cerevisiae*, the expression of *asgs* is turned on by the binding of the Mcm1 gene-regulatory factor to a characteristic stretch of DNA located upstream of each *asg* coding region³ (Fig. 1). But in *C. albicans*, in addition to an Mcm1-binding site, this regulatory motif also turned out to have a binding site for the *a2* protein. Comparing the upstream regulatory motifs governing *asg* regulation from several additional yeasts identified two lineages: *C. albicans* and its close relatives possess motifs with binding sites for both Mcm1 and *a2*, whereas *S. cerevisiae* and related species have motifs with only Mcm1-binding sites.

Although *S. cerevisiae* has lost *a2* and thus the ability to control the activation of its *asgs*, it has gained the ability to repress *asg* expression through the α 2 and Mcm1 proteins. Specifically, Tsong *et al.*² show that the upstream regulatory motif of the *S. cerevisiae* lineage seems to have acquired two α 2-binding sites. Furthermore, the domain of α 2 that interacts with Mcm1 is similar among all the species with the *S. cerevisiae*-like regulatory architecture,

but is not conserved in species with the *C. albicans*-like architecture. It is probable that specific mutations in the Mcm1-interaction domain of α 2 enabled the *S. cerevisiae* lineage to acquire the ability to repress *asgs*.

Intriguingly, a third yeast lineage (*Kluyveromyces lactis* and its relatives) exhibits tell-tale signs of an intermediate architecture between those exemplified by *C. albicans* and *S. cerevisiae*. For example in *K. lactis*, *a2* participates in *asg* activation (as in *C. albicans*), but α 2 interacts with Mcm1 to suppress *asgs* in α cells (as in *S. cerevisiae*). *K. lactis* diverged from *S. cerevisiae* after their joint common ancestor split from *C. albicans*, so could it be that it has maintained a 'transitional' regulatory architecture? Although the weight of evidence supports this argument, it has yet to be shown that the *K. lactis* *a2* and α 2 proteins do actually bind to the putative *asg* binding sites.

How could this transition from positive to negative regulation of *asgs* occur? From a theoretical standpoint, because natural selection is operating on phenotypes, 'neutral' changes in the underlying genotypes that do not affect the phenotype are not screened by the sieve of selection and can become fixed in natural populations^{4,5}. Tsong and colleagues' evidence² suggests not only that this rewiring of the mating circuit could have happened in a neutral fashion, but also that it may have required only a few mutational steps. Indeed, increasing the number of A and T bases around the Mcm1-binding site in *S. cerevisiae* can allow Mcm1 to activate *asgs* in the absence of *a2* (ref. 6). Moreover, the *a2*- and α 2-binding sites in *S. cerevisiae* are remarkably similar, so the transition from positive (by *a2*) to negative (by α 2) regulation of *asgs* might have occurred by only a handful of mutations.

Interestingly, *C. albicans* possesses an extra environment-dependent layer of gene regulation in mating-type control that *S. cerevisiae*

does not have and that probably represents an adaptation to its pathogenic lifestyle⁷. This additional control level is unlikely to have been present in the common ancestor of *C. albicans* and *S. cerevisiae*, suggesting that some of the observed differences in regulatory architecture must have been the direct result of natural selection.

As for the bigger picture, several studies indicate that genotypic divergence in the face of phenotypic conservation may be a rather common, but underappreciated, evolutionary theme^{4,5}. Examples of such 'developmental system drift' range from ribosomal transcriptional modules in the same yeast lineage⁸, to the genetic basis of tooth formation in vertebrates⁹, and regulatory evolution in fruitflies¹⁰, although it is still unclear whether all this genotypic divergence was acquired through neutral evolution.

Examination of the mating circuits of other yeast species promises further evolutionary delights. For example, *Pichia angusta* seems to have lost the *MATa2* gene independently¹¹, and several of the genes involved in *C. albicans* mating are absent from its close relative *Candida parapsilosis*¹². Finally, considering the technical difficulties associated with the study of mating in species such as *K. lactis* and the tremendous power offered by the *S. cerevisiae* model system, perhaps a fruitful alternative may be to reconstruct the mating circuits of the direct ancestors of *S. cerevisiae* (is this the birth of palaeoregulomics?). For example, engineering a *S. cerevisiae* strain with a *C. albicans*-like or a *K. lactis*-like architecture would allow an analysis of how long-lost ancestors may have regulated mating. Competition experiments of these 'ancestral' strains with modern-day ones would settle the question of whether such a remarkable shift in the architecture of a regulatory circuit could have been pulled off in a neutral fashion. ■

Antonis Rokas is in the Microbial Genome Analysis and Annotation Group, The Broad Institute of MIT and Harvard, 7 Cambridge Center, Massachusetts 02142, USA.
e-mail: arokas@mit.edu

- Carroll, S. B. *PLoS Biol.* **3**, e245 (2005).
- Tsong, A. E., Tsuchi, B. B., Li, H. & Johnson, A. D. *Nature* **443**, 415–420 (2006).
- Acton, T. B., Mead, J., Steiner, A. M. & Vershon, A. K. *Mol. Cell. Biol.* **20**, 1–11 (2000).
- True, J. R. & Haag, E. S. *Evol. Dev.* **3**, 109–119 (2001).
- Weiss, K. M. & Fullerton, S. M. *Theor. Popul. Biol.* **57**, 187–195 (2000).
- Acton, T. B., Zhong, H. & Vershon, A. K. *Mol. Cell. Biol.* **17**, 1881–1889 (1997).
- Tsong, A. E., Miller, M. G., Raisner, R. M. & Johnson, A. D. *Cell* **115**, 389–399 (2003).
- Tanay, A., Regev, A. & Shamir, R. *Proc. Natl Acad. Sci. USA* **102**, 7203–7208 (2005).
- Kawasaki, K., Suzuki, T. & Weiss, K. M. *Proc. Natl Acad. Sci. USA* **102**, 18063–18068 (2005).
- Ludwig, M. Z., Bergman, C., Patel, N. H. & Kreitman, M. *Nature* **403**, 564–567 (2000).
- Butler, G. *et al.* *Proc. Natl Acad. Sci. USA* **101**, 1632–1637 (2004).
- Logue, M. E., Wong, S., Wolfe, K. H. & Butler, G. *Eukaryot. Cell* **4**, 1009–1017 (2005).

CONDENSED-MATTER PHYSICS

Coherent questions

David Snoke

Bose–Einstein condensation occurs when many particles enter into the same, coherent quantum state, and is now claimed to occur in various systems of ‘quasiparticles’ in solids. But is it the right term to use here?

The basic theory of the phenomenon known as Bose–Einstein condensation is well understood. When the temperature of a gas of integer-spin particles, or bosons, is low enough, thermodynamics causes a significant fraction of them to spontaneously enter a single quantum state. These particles form the condensate, and they act collectively as a coherent, classical wave.

In this issue, Kasprzak *et al.* (page 409)¹ and Demokritov *et al.* (page 430)² extend the concept of Bose–Einstein condensation to two different types of quasiparticles — discrete quanta of energy that can be treated as real particles — in solids. These papers, together with another published two years ago³, raise questions of terminology. First, two of the systems^{1,3} are two-dimensional. But wasn't it proved long ago that Bose–Einstein condensation is forbidden in two dimensions? Second, in one of the experiments¹, the quasiparticles live for just a few picoseconds. Can we really talk of an equilibrium condensation in such a system? Third, in two of the systems^{1,2}, a coherent field is driving the system, and the number of particles is not conserved. Under these circumstances, is the condensation really a thermodynamic phase transition? Or, with all these objections, is it legitimate to say that we now have another class of condensates? Should we put the Bose–Einstein condensation of quasiparticles in solids on the list along with condensates of atoms in optical traps and superfluid helium?

Rather than haggling over the exact meaning of the term Bose–Einstein condensation, we can adopt a more general concept that allows us to use the same language to talk about many systems. This concept is the spontaneous emergence of coherence. When helium condenses into a superfluid at low temperatures, it can be described as a strongly interacting, spontaneously coherent system. Atoms in traps, which form the classic Bose–Einstein condensate, are an example of a weakly interacting, spontaneously coherent system. Superconductivity stems from the spontaneous coherence of paired-up electrons known as Cooper pairs. The onset of coherent emission from a laser is also an example of spontaneous coherence, although in this case the coherence does not occur through a thermodynamic phase transition.

This spontaneous coherence can occur in two-dimensional systems just as well as in three dimensions. Experiments with liquid helium on surfaces, for example, have shown that two-dimensional helium can be superfluid, just like bulk helium⁴. But in two dimensions,

the coherence length cannot be infinite⁵: over large distances, fluctuations will cause regions to go out of phase with one another. Such fluctuations can also occur in three-dimensional systems, but there is no upper bound to the coherence length⁶.

When the system is finite in two dimensions — in a trap, for instance — the distinction between a two-dimensional and a three-dimensional condensate is moot, as both have an upper bound for the coherence length dictated by the system's size. Kasprzak *et al.*¹, for example, created polaritons in a finite cloud in a two-dimensional structure using a focused laser. (Polaritons are quasiparticles formed from electronic excitations strongly coupled to photons.) The authors argue that the coherence length in this case is comparable to the size of the cloud of polaritons. An alternative experimental approach is to create a trap to confine polaritons to a finite region using an external force⁷, and initial results with trapped polaritons in gallium arsenide semiconductors also show spontaneous coherence.

Can quasiparticles with finite lifetime — whose numbers are not conserved — undergo Bose–Einstein condensation? The consensus on this issue, reached about a decade ago^{8,9}, is that if the lifetime of the particles is much longer than the time they need to scatter with each other, condensation is possible, although there may not be time to measure the resulting superfluidity¹⁰.

In practice, atoms in optical traps do have a finite lifetime — they evaporate or form molecules — but this does not prevent condensation. For quasiparticles in solids, the situation is no different, but the timescales are shorter. Although the picosecond lifetime of Kasprzak and colleagues' polaritons¹ might seem short, the particles can interact even faster. The evidence comes from the distribution function of the particles' energy, which fits a Maxwell–Boltzmann distribution (meaning that the curve tails off at high energy in a way that fits a straight line when plotted semi-logarithmically) when the particles are not condensed. This behaviour is clearly observed in the polariton experiments when the particles are near condensation¹. When the particles condense, presumably they interact even faster. In the same way, in Demokritov and colleagues' study², the number of magnons — quasiparticles representing collective spin excitations in a solid — can be approximately conserved on sub-microsecond timescales. If they interact on even shorter

timescales, they can undergo condensation.

As spontaneous coherence is so essential to the phenomenon of Bose–Einstein condensation, to prove that one has a condensate, one must demonstrate two things: first, that there is coherence, and second, that this coherence is spontaneous. Experiments showing interference between two condensates¹¹ are generally regarded as more convincing evidence of coherence, and therefore condensation, than observations of many particles seemingly in the ground state. Experimentally, with an energy distribution alone, it is difficult to tell whether particles really are in the same quantum state, or just close to it.

Direct evidence of spontaneous coherence of quasiparticles in solids has been hard to come by. Demokritov *et al.*² demonstrate a build-up of a population of magnons near the ground state, but present no direct test of coherence. In earlier experiments³, coherence among excitons was deduced indirectly from measurements of their current. Kasprzak *et al.*¹, however, do provide direct evidence of coherence among exciton polaritons, both in the interference and in the spontaneous linear polarization of light emitted by the system.

That the coherence should be spontaneous is essential. Many systems can be coherent if driven by another coherent source: any loudspeaker, for example, generates a coherent state of long-wavelength phonons (sound quanta). Calling this a 'driven condensate' would, however, seem to contradict the spirit of the term 'condensate' as a spontaneous thermodynamic phenomenon. Equally, the coherence of polaritons directly coupled to a coherent laser beam¹² is not generally accepted as condensation, although the phenomenon has great promise for the development of nonlinear devices for optical switching^{13,14}. In Kasprzak and colleagues' case¹, as in earlier attempts¹⁵, the laser that creates the polaritons is not directly coupled to the condensate; numerous phonons are also emitted, which, one presumes, scramble any phase information from the laser. Thus, the coherence in the polariton condensate must be spontaneous, and indeed, there is a clear change from incoherent to coherent light emission at the transition point in these experiments.

The polariton condensate¹ is a pumped system that emits coherent light. So is it fundamentally different from a laser? Some have given it the name 'polariton laser'¹⁵. Fundamentally, both are examples of spontaneous coherence. In the case of a laser, however, coherent emission occurs only when enough energy is pumped into a system so that an excited electronic state acquires a population far greater than that of the ground state. In the case of a polariton condensate, the thermodynamics of bosons drives the transition. This means that the coherent light emission occurs without a population inversion¹⁶. This may lead to very low-power coherent light emitters in the future.

After steady work for two decades, the field of the condensation of quasiparticles in solids

is now blossoming. Besides the examples discussed^{1–3}, experiments continue on trapping excitons in coupled quantum wells¹⁷, on excitons in bulk semiconductors¹⁸, and on tripletons in magnetic insulators¹⁹. The appeal of quasiparticles in solids as systems to observe condensation is, in part, their light mass. The smaller the mass, the higher the critical temperature at which condensation occurs, and there is no reason why condensation of one of these quasiparticles cannot occur at room temperature. In fact, Demokritov and colleagues' experiments with magnons² supply evidence that this might already have been observed. Some of these schemes of quasiparticle condensation also, intriguingly, imply superconductivity²⁰.

We now have several examples of systems with evidence of spontaneous coherence caused by a thermodynamic phase transition. Some people may not want to call certain cases Bose–Einstein condensation, but these systems collectively represent a new frontier in coherent phenomena. ■

David Snoke is in the Department of Physics and

Astronomy, G10 Allen Hall, 3941 O'Hara Street, Pittsburgh, Pennsylvania 15260, USA.

e-mail: snoke+@pitt.edu

1. Kasprzak, J. *et al.* *Nature* **443**, 409–414 (2006).
2. Demokritov, S. O. *et al.* *Nature* **443**, 430–433 (2006).
3. Eisenstein, J. P. & MacDonald, A. H. *Nature* **432**, 691–694 (2004).
4. Bishop, D. J. & Reppy, J. D. *Phys. Rev. Lett.* **40**, 1727–1730 (1978).
5. Nelson, D. in *Phase Transitions and Critical Phenomena* (eds Domb, C. & Lebowitz, J. L.) 11–31 (Academic, New York, 1983).
6. Griffin, S. *Excitations in a Bose-condensed Liquid* 47–53 (Cambridge Univ. Press, 1993).
7. Balili, R. *et al.* *Appl. Phys. Lett.* **88**, 031110 (2006).
8. Snoke, D. W. & Wolfe, J. P. *Phys. Rev. B* **39**, 4030–4037 (1989).
9. Stoof, H. T. C. *Phys. Rev. A* **45**, 8398–8406 (1992).
10. Szymanska, M. H. *et al.* *Phys. Rev. Lett.* **96**, 230602 (2006).
11. Andrews, M. R. *et al.* *Science* **275**, 637–641 (1997).
12. Kavokin, A. & Malpeuch, G. *Cavity Polaritons* 147–181 (Elsevier, Amsterdam, 2003).
13. Kundermann, S. *et al.* *Phys. Rev. Lett.* **91**, 107402 (2003).
14. Savvidis, P. G. *et al.* *Phys. Rev. Lett.* **84**, 1547–1550 (2000).
15. Deng, H., Weihs, G., Snoke, D., Bloch, J. & Yamamoto, Y. *Proc. Natl Acad. Sci. USA* **100**, 15318–15323 (2003).
16. Imamoglu, A. & Ram, J. R. *Phys. Lett. A* **214**, 193–196 (1996).
17. Vörös, Z. *et al.* *Phys. Rev. Lett.* **97**, 016803 (2006).
18. Fröhlich, D. *et al.* *Solid State Comm.* **134**, 139–142 (2005).
19. Ruegg, N. *et al.* *Nature* **423**, 62–65 (2003).
20. Yudson, V. I. *Phys. Rev. Lett.* **77**, 1564–1567 (1996).

AGEING

Balancing regeneration and cancer

Christian M. Beausejour and Judith Campisi

The proliferation of cells must balance the longevity assured by tissue renewal against the risk of developing cancer. The tumour-suppressor protein p16^{INK4a} seems to act at the pivot of this delicate equilibrium.

Tissue repair and regeneration are essential for longevity in complex animals, and often depend on the proliferation of unspecialized cells known as stem or progenitor cells. In many tissues, the regenerative capacity of such cells declines with age, and it is thought that this decline drives many age-related symptoms¹. But stem/progenitor-cell proliferation is a double-edged sword. Although it ensures tissue repair and regeneration, it also puts tissues at risk of hyperproliferative diseases, the most deadly of which is cancer. This risk is mitigated by tumour-suppressor mechanisms, which either eliminate potential cancer cells by programmed cell death (apoptosis) or prevent their proliferation, often by permanently halting the cell division cycle (senescence). Therefore, the benefits to longevity afforded by stem/progenitor-cell proliferation might be compromised by mechanisms that prevent life-threatening cancer². Three papers^{3–5} in this issue now find that this is indeed the case^{3–5}.

In mammals, the p16^{INK4a} protein inhibits cell-cycle progression and induces cellular senescence⁶, and its expression rises with age in many tissues, as does the accumulation of dysfunctional senescent cells^{7,8}. Janzen *et al.*³

(page 421) find that p16^{INK4a} levels increase with age in mouse 'haematopoietic' stem cells (HSCs) derived from the bone marrow. Other bone-marrow cells did not express p16^{INK4a} in an age-dependent manner, so p16^{INK4a} is a biomarker of ageing HSCs in the bone marrow.

But is this rise in p16^{INK4a} expression biologically important? HSCs give rise to mature white blood cells. They can reconstitute these immune cells after being transplanted into mice that have been irradiated, and so have no HSCs. This reconstitution ability declines with age. Janzen *et al.*³ report that young mice (2–3 months old) that lack the p16^{INK4a} protein (p16^{INK4a}−/− mice) have similar numbers of HSCs to normal, 'wild-type' mice of the same age. Moreover, the wild-type and p16^{INK4a}−/− HSCs are equally able to reconstitute an immune system after transplantation.

Later in life (14–20 months), however, the p16^{INK4a}−/− mice had significantly more HSCs than wild-type mice. The older p16^{INK4a}−/− HSC populations also had more dividing cells and were better able to reconstitute an immune system than were wild-type HSCs from mice of the same age. So lack of p16^{INK4a} slows the age-associated decline in HSC function. Unexpectedly, however, the young p16^{INK4a}−/− HSCs were less effective at reconstituting an immune

system than were wild-type HSCs of either age. These findings suggest that, in young animals, p16^{INK4a} prevents premature HSC exhaustion under the intense proliferative demand following transplantation. But in older animals, p16^{INK4a} restricts HSC proliferation both under normal steady-state conditions and after transplantation.

Krishnamurthy *et al.*⁴ (page 453) studied a very different tissue in mice — the pancreas, which also shows an age-associated decline in function (here measured by insulin production) and in regenerative capacity. Expression of p16^{INK4a} also increased in the pancreas during ageing (3–4 months compared with 16–20 months). This rise was confined mainly to the pancreatic islets, the cell clusters that contain insulin-producing β-cells and their progenitors.

The authors report two lines of evidence that p16^{INK4a} limits islet-cell proliferation during ageing. First, mice genetically engineered to overexpress p16^{INK4a} at young ages — to levels found in older wild-type animals — showed reduced islet-cell proliferation at all ages. Second, in mice deficient in p16^{INK4a}, the age-associated decline in islet-cell proliferation was markedly diminished. When older wild-type mice were given a toxin that selectively kills β-cells, they developed diabetes that was eventually fatal. But, importantly, older mice deficient in p16^{INK4a} developed only mild diabetes when exposed to the toxin. Recovery from the toxin requires β-cell proliferation and the regeneration of functional islets. After toxin treatment, the p16^{INK4a}−/− mice had better islet-cell regeneration, greater insulin production and reduced blood-glucose levels than did wild-type mice. These differences occurred only between mice from the older age group, suggesting that the age-associated rise in p16^{INK4a} impairs islet-cell regeneration and function, presumably by inhibiting the proliferation of β-cells and/or their progenitors.

In the third paper, Molofsky *et al.*⁵ (page 448) link p16^{INK4a} expression in mice to decreased formation of neurons originating in the forebrain 'subventricular zone' (SVZ). As with HSCs and the pancreas, p16^{INK4a} expression increased in the SVZ with ageing (from 2 to 24 months). Moreover, SVZ cell proliferation declined *in vivo* with age, as did the number of stem/progenitor cells that form clusters that can proliferate and retain the ability to differentiate in culture. Deficiency of p16^{INK4a} partially mitigated both of these traits in older animals, but there was no effect in young animals. Lack of p16^{INK4a} also slowed the age-associated decline in the formation of neurons in the olfactory bulb. Olfactory neurons are known to originate in the SVZ. Notably, p16^{INK4a} deficiency slowed the age-dependent loss of SVZ stem cells (which divide continually but slowly), rather than stimulating the rapid proliferation of progenitor cells (the progeny of stem cells).

These findings suggest that p16^{INK4a} causes a substantial part of the age-associated decline in

*This article and the papers concerned^{3–5} were published online on 6 September 2006.

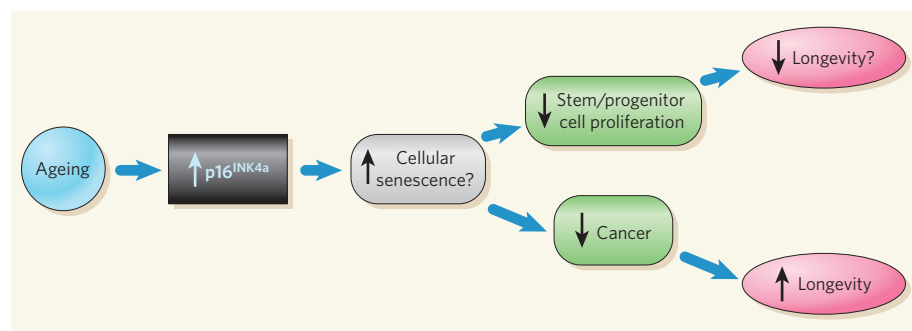


Figure 1 | Dual activities of p16^{INK4a} during ageing. Ageing causes an increase in p16^{INK4a}, a potent tumour suppressor that promotes longevity by suppressing the development of cancer. Three papers show that p16^{INK4a} suppresses the proliferation of stem or progenitor cells in the bone marrow, pancreas and brain^{3–5}. The mechanisms that induce p16^{INK4a} during ageing are not known (black box). Once expressed, p16^{INK4a} causes cellular senescence (grey box), a permanent growth-arrested state, which may be the mechanism by which this protein suppresses the proliferation of stem/progenitor cells. The decline in stem/progenitor-cell proliferation compromises tissue regeneration and repair, which is likely to reduce longevity.

the potential to form new SVZ neurons. However, p16^{INK4a} deficiency did not prevent age-associated declines in cell proliferation in the dentate gyrus region of the brain or the enteric nerves in the gut, so different mechanisms may drive the ageing of stem/progenitor cells in other parts of the nervous system.

These three papers uncover a novel role for the p16^{INK4a} tumour suppressor in promoting ageing (Fig. 1), a role shared by the p53 tumour suppressor^{9,10}. But they also raise many questions. Does p16^{INK4a} drive stem/progenitor-cell

ageing by inducing an irreversible senescence arrest, a reversible quiescent state or another mechanism? What causes the age-dependent rise in p16^{INK4a} expression? Is it induced by hormones such as those that regulate the insulin/IGF pathway during ageing¹¹? Or is it caused by stress or damage signals within the cells? Given that p16^{INK4a} deficiency only partly mitigates most of the ageing effects studied, what other mechanisms cause stem/progenitor-cell ageing? Finally, how important is the ageing of cells, in this case stem/progenitor cells, for the longevity

of an organism? Does it depend on the type or level of stress that the organism experiences?

Answers to these questions might clarify whether drugs that blunt p16^{INK4a} expression or activity will ameliorate certain age-related diseases. Whatever the answers, it is important to remember that p16^{INK4a}-deficient mice frequently succumb to cancer in mid-life (1.5–2 years). We will therefore need to know much more about the regulators and effectors of p16^{INK4a} before these remarkable findings can be harnessed to make effective longevity-promoting therapies.

Christian M. Beausejour is at the Centre de recherche pédiatrique, Hôpital Sainte-Justine, Université de Montréal, 3175 chemin de la Côte-Sainte-Catherine, Montréal H3T 1C5, Canada. Judith Campisi is at the Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, California 94720, USA, and the Buck Institute for Age Research. e-mail: jcampisi@lbl.gov

1. Krtolica, A. *Int. J. Biochem. Cell Biol.* **37**, 935–941 (2005).
2. Campisi, J. *Nature Rev. Cancer* **3**, 339–349 (2003).
3. Janzen, V. *et al. Nature* **443**, 421–426 (2006).
4. Krishnamurthy, J. *et al. Nature* **443**, 453–457 (2006).
5. Molofsky, A. V. *et al. Nature* **443**, 448–452 (2006).
6. Collins, C. J. & Sedivy, J. M. *Ageing Cell* **2**, 145–150 (2003).
7. Campisi, J. *Cell* **120**, 1–10 (2005).
8. Krishnamurthy, J. *et al.* **114**, 1299–1307 (2004).
9. Tyner, S. D. *et al. Nature* **415**, 45–53 (2002).
10. Maier, B. *et al. Genes Dev.* **18**, 306–319 (2004).
11. Bartke, A. *Endocrinology* **146**, 3718–3723 (2005).

CLIMATE CHANGE

A nasty surprise in the greenhouse

Jos Lelieveld

The Kyoto Protocol aims to reduce emissions of greenhouse gases such as methane. But it seems that the fall in human-induced methane emissions in the 1990s was only transitory, and atmospheric methane might rise again.

Methane is a potent greenhouse gas — per molecule, more than 20 times as powerful as carbon dioxide¹. Moreover, when methane emissions rise, so too does the concentration of the pollutant ozone in the troposphere, the lowest layer of Earth's atmosphere². Methane also consumes hydroxyl radicals, whose oxidative effects are essential to atmospheric cleansing. On page 439 of this issue³, Bousquet *et al.* recount the results of an international effort to measure atmospheric methane concentrations and combine these data with a computer model of atmospheric chemistry and transport. The bad news is that the slowdown in global methane emissions in the past few decades was only temporary: reports of the emissions' control have been exaggerated.

At present, about two-thirds of global methane comes from anthropogenic sources, and most emissions occur in the Northern Hemisphere (Fig. 1, overleaf). Of naturally produced

methane, the largest proportion stems from bacteria in wetlands that produce the gas when decomposing organic material. The growth rate of atmospheric methane was more than 10% per decade before 1980, but by the 1990s it had dropped to nearly zero (Fig. 2, overleaf)⁴. Bousquet and colleagues³ compute the global methane source distribution, especially its variability over recent decades. This is a rather controversial issue, as it is difficult to determine whether this variability should be attributed to fluctuations in the sources or in the sinks; the sink mechanisms are dominated by the good offices of the atmospheric hydroxyl radicals⁵.

The authors used a so-called inversion modelling technique, which starts from observed concentrations at Earth's surface and back-calculates using models of transport and loss processes to optimize source estimates. The measurements stem from a global network

of monitoring stations, and include isotope data (in particular, the relative proportion of carbon-13) that provide an additional clue as to what methane came from where. Methane from biomass burning, fossil-fuel-related sources and bacterial processes have distinct isotopic signatures; methane emissions from wetlands, for example, are substantially depleted in carbon-13.

The approach is novel because the model computations optimized both methane emissions and methane loss through hydroxyl oxidation. The crux of the findings is that fluctuations of natural emissions, in particular by wetlands in the tropics, are a dominant factor in the variability of methane from year to year. These emissions are in turn sensitive to meteorological parameters: during dry periods, methane flux from wetlands is depressed.

Thus, during the most recent part of the analysis period — from 1999 onward — extended droughts have reduced natural methane emissions. This has concealed the fact that anthropogenic emissions have resumed their increase, an increase perhaps associated with the accelerating use of fossil fuels by booming Asian economies. Continued monitoring of atmospheric methane, and especially its relation to wetland inundation and drying, will be needed to substantiate this prediction.

Bousquet and colleagues' study³ is not incompatible with the recent suggestion that

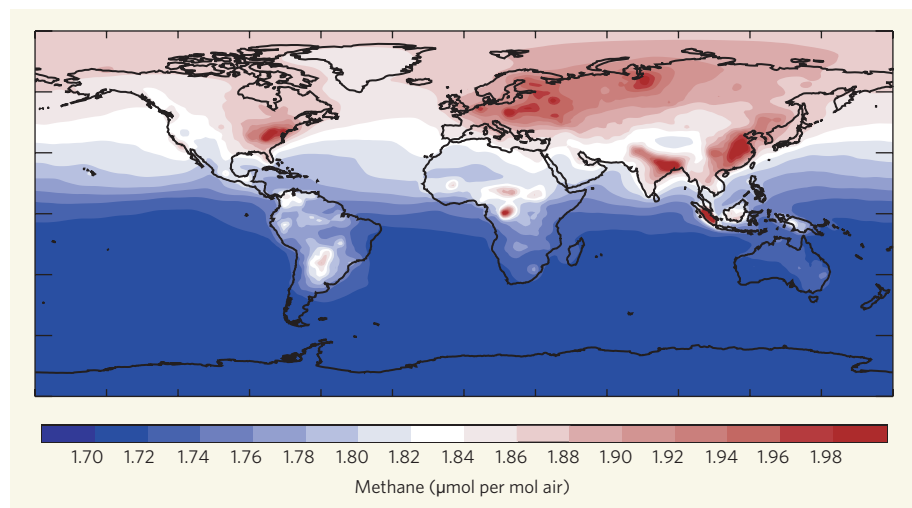


Figure 1 | Methane hotspots. Average methane mixing ratios in the boundary layer (the layer of the atmosphere in immediate contact with Earth's surface) in 2003, calculated with a chemistry–transport model. The atmospheric lifetime of methane is almost a decade, so it disperses globally. Regions of strong emissions are nevertheless manifest, leading to the largest variability in the Northern Hemisphere and an inter-hemispheric difference of 5–10%. The recently proposed release of methane by terrestrial vegetation is not included, as its magnitude is still uncertain. (Figure courtesy S. Houweling⁵.)

terrestrial vegetation is a strong methane source⁶. It sounds paradoxical that adding a large new source such as this, which contributes 30% of the global methane flux to the model, and decreasing the others proportionately can be done without fundamentally changing the inversion calculations. But the combination of the relatively strong meridional methane gradient (Fig. 2) and too few measurement stations in the tropics makes constraining the size of single-source categories such as forests and wetlands tricky.

Further confirmation of a large vegetation source comes from satellite measurements showing that methane concentrations are enhanced over the tropical rainforests⁷. It is remarkable not only that this large new methane source has just been discovered, but also that mechanisms for producing methane deviate from the known anaerobic formation by microbes. The role of oxygen in methanogenesis, including its application in renewable energy production, should receive much more attention in the coming years.

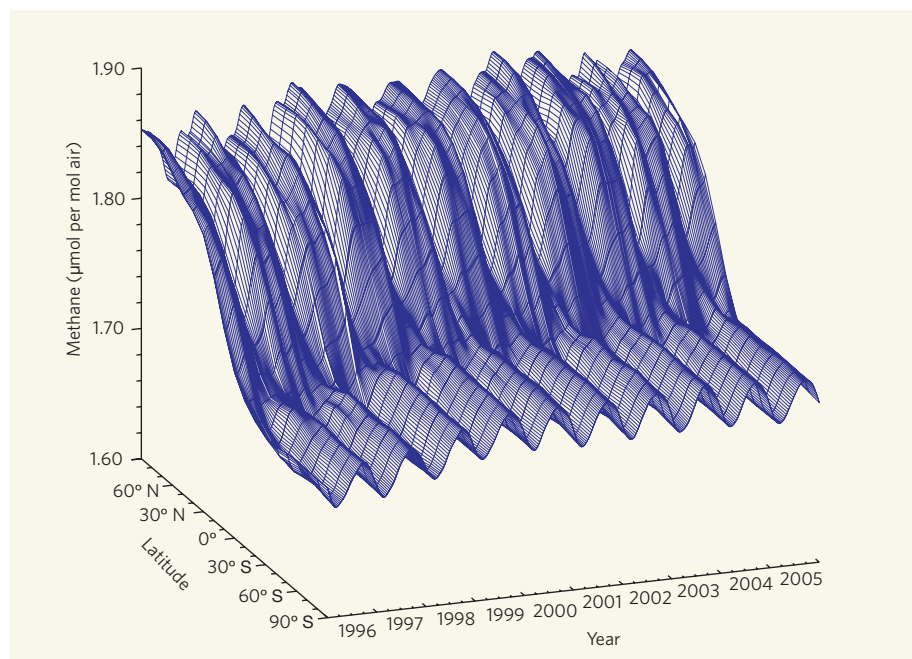


Figure 2 | Meridional slide. The latitudinal methane distribution in the marine boundary layer over the past 10 years, based on measurements in the air-sampling network of the Earth System Research Laboratory of the US National Oceanic and Atmospheric Administration. Annual fluctuations and the higher methane concentrations at higher northern latitudes are clear, but over the period surveyed, the year-on-year trend is small. Bousquet *et al.*³ suggest that recent dry years have led to a drop in natural methane emissions, masking a rise in emissions induced by human activities. (Figure courtesy E. Dlugokencky⁴.)

Atmospheric methane is a factor in the amplification of climate change, because the amount of methane released by wetlands and vegetation responds sensitively to temperature and moisture conditions. This establishes a positive-feedback mechanism that has contributed to rapid climate shifts during the last glacial cycle⁸. But Bousquet and colleagues' analysis³ also allows for a negative-feedback mechanism, put in place by atmospheric chemistry. In dry periods of reduced methane emissions, methane removal by hydroxyl radicals also decreases. The dryness aggravates vegetation fires, which release large amounts of carbon monoxide, and this pollutant gas also consumes hydroxyl. With less hydroxyl around, less methane is broken down, and the decrease in methane concentration is not as large as might be expected.

This phenomenon is not natural, however: most fires are ignited by humans. Furthermore, vast amounts of methane are deposited as hydrates in permafrost regions and in marine sediments. It is as yet unclear to what extent the melting of permafrost and increasing ocean temperature will affect these methane reservoirs, destabilize the hydrates and exacerbate greenhouse warming. These processes influence both atmospheric methane and hydroxyl, and are potentially important feedback mechanisms that require further research.

It will be both essential and difficult to control greenhouse-gas emissions and verify nationally reported inventories. Human-induced emissions scale with fossil-fuel consumption, and the global yearning for energy, especially in nations that do not recognize the Kyoto Protocol on climate change, gives rise to concern about climate change. The uncertainty range associated with climate projections¹ implies that large changes will be as likely as modest ones. Such large and possibly disruptive climate changes ask for short-term solutions.

Unfortunately, the response times of most greenhouse gases in the atmosphere and their climatic effects are slow — decades to centuries. Measures to control methane emissions should be placed high on the agenda because they, in contrast, become effective within a few years. In a situation where climate change is accelerating, there is no time to lose: we need effective solutions, and we must act fast. ■

Jos Lelieveld is at the Max-Planck-Institut für Chemie, Johann-Joachim-Becher-Weg 27, 55128 Mainz, Germany.
e-mail: lelieveld@mpch-mainz.mpg.de

1. Ramaswamy, V. *et al.* in *Climate Change 2001: The Third Assessment Report of the Intergovernmental Panel on Climate Change* (eds Houghton, J. T. *et al.*) 349–416 (Cambridge Univ. Press, 2001).
2. Lelieveld, J., Crutzen, P. J. & Dentener, F. J. *Tellus* **50B**, 128–150 (1998).
3. Bousquet, P. *et al.* *Nature* **443**, 439–443 (2006).
4. Dlugokencky, E. J. *et al.* *Geophys. Res. Lett.* **30**, 1992 (2003).
5. Houweling, S. *et al.* *J. Geophys. Res.* **104**, 26137–26160 (1999).
6. Keppler, F. *et al.* *Nature* **439**, 187–191 (2006).
7. Frankenberg, C. *et al.* *Science* **308**, 1010–1014 (2005).
8. Brook, E. J. *et al.* *Glob. Biogeochem. Cycles* **14**, 559–572 (2000).

BRIEF COMMUNICATIONS

Silk-like secretion from tarantula feet

An unsuspected attachment mechanism may help these huge spiders to avoid catastrophic falls.

Spiders spin silk from specialized structures known as abdominal spinnerets — a defining feature of the creatures^{1,2} — and this is deployed to capture prey, protect themselves, reproduce and disperse. Here we show that zebra tarantulas (*Aphonopelma seemanni*) from Costa Rica also secrete silk from their feet to provide adhesion during locomotion, enabling these spiders to cling to smooth vertical surfaces. Our discovery that silk is produced by the feet provides a new perspective on the origin and diversification of spider silk.

Spider feet have a 'dry' attachment system that relies on van der Waals forces generated by thousands of spatulate hairs³. Additionally, small distal claws enhance adhesion to rough surfaces by mechanically interlocking with the substrate. We have discovered that the tarantula *A. seemanni* (Fig. 1) has a third attachment mechanism, which depends on fibres exuded from nozzle-like structures on its feet. These fibrous secretions (Fig. 2) function as silken tethers and, when laid down on glass plates, appear as 'footprints' that consist of dozens of fibres with diameters of 0.2–1.0 micrometres and lengths of 100–2,500 micrometres (for details of analyses, see supplementary information).

Individual tarsal silk fibres often start as a flattened plaque. This seems to be secreted as a viscous fluid that solidifies, gluing the thread to the substrate. The function and morphology of the tarantula's tarsal silk resembles the adhesive agent produced by the spinnerets of the spider *Antrodiaetus unicolor*⁴, as well as the attachment (pyriform) silk that many spiders use to cement their draglines to substrates⁵.

We induced *A. seemanni* to walk on vertical glass surfaces in order to observe the contact mechanics of this challenging locomotion. When walking up vertical planes, the spider attached only the distal parts of its tarsi to the substrate. As it started to slip down the glass, silk produced by the tarsal spigots on all four pairs of legs arrested the spider's descent and allowed it to remain attached to the vertical surface. The spider's feet were positioned such that the silk-producing spigots were in contact with the glass, while the dense setae in adjacent regions were held off the surface.

Our discovery of secreted tarsal silk forces a reconsideration of the evolution of spider silks^{1,5,6}. Depending on its distribution across spider phylogeny, tarsal synthesis of silk could represent the ancestral condition, with silk



Figure 1 | Costa Rican zebra tarantula, *Aphonopelma seemanni*. Female, anterior view. Scale bar, 5 mm.

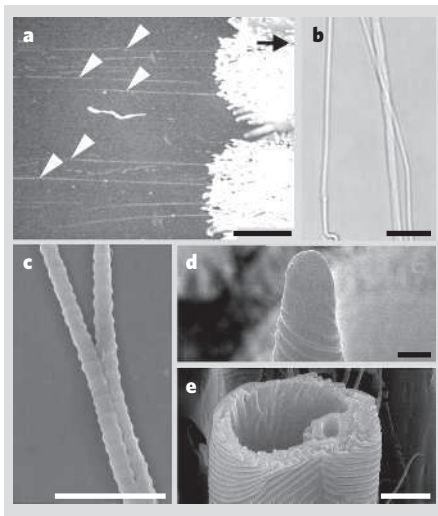


Figure 2 | Tarsal silk production by the spider *Aphonopelma seemanni*. **a**, Fibres left behind (arrowheads) by a spider sliding down a vertical glass surface. Black arrow indicates direction of sliding. The spherical structures are the distal part of the tarsus (scopula), covered with hairs and spigots. **b**, Traces left by the tarsus of a spider walking on a coverslip. **c**, Single fibres observed by cryoscanning electron microscopy. **d**, Tip of a tarsal spigot with the opening obstructed by silk. **e**, Tarsal spigot broken near the base. Scale bars: **a**, 500 μ m; **b**, 10 μ m; **c**, 1 μ m; **d**, 2 μ m; **e**, 5 μ m.

production from abdominal spinnerets evolving later. Alternatively, because the tarantula clade (Theraphosidae) is a species-rich group that includes the largest known spiders⁷, tarsal production of silk may have evolved independently as a key innovation to enhance locomotor ability and avert catastrophic falls.

Both evolutionary hypotheses are consistent with the homology of legs and spinnerets as arthropod appendages^{1,5,8}. Regardless of whether tarsal silk production is ancestral or secondarily derived, the silk-producing

apparatus of spiders seems to be controlled by developmental modules that can be expressed in a variety of body parts.

Investigation of the genes involved in tarsal silk production should resolve whether the original function of spider silk was to increase traction or whether it was later co-opted for that purpose. Spinneret silk proteins are encoded by a gene family that has evolved through a series of gene duplications and subsequent modifications for particular tasks^{9–11}. If tarsal silks belong to the same gene family, then comparison of tarsal and spinneret silks should help our understanding of the ancestral function and composition of spider silk.

Stanislav N. Gorb*†, **Senta Niederegger***†‡, **Cheryl Y. Hayashi§**, **Adam P. Summers||**, **Walter Vötsch†**, **Paul Walther¶**

*Evolutionary Biomaterials Group, Max Planck Institute for Metals Research, 70569 Stuttgart, Germany

e-mail: s.gorb@mf.mpg.de

†Max Planck Institute for Developmental Biology, 71076 Tübingen, Germany

‡Institute of Forensic Medicine, Friedrich Schiller University of Jena, 07743 Jena, Germany

§Department of Biology, University of California, Riverside, California 92521, USA

||Ecology and Evolutionary Biology, University of California, Irvine, California 92697, USA

¶Electron Microscopy Department, University of Ulm, 89069 Ulm, Germany

- Shultz, J. W. *Biol. Rev.* **62**, 89–113 (1987).
- Vollrath, F. & Knight, D. P. *Nature* **410**, 541–548 (2001).
- Arzt, E., Gorb, S. & Spolenak, R. *Proc. Natl Acad. Sci. USA* **100**, 10603–10606 (2003).
- Palmer, J. M., Coyle, F. A. & Harrison, F. W. *J. Morphol.* **174**, 269–274 (1982).
- Bristowe, W. S. *Proc. Zool. Soc. Lond.* **103**, 1015–1057 (1932).
- Craig, C. L. *Annu. Rev. Entomol.* **42**, 231–267 (1997).
- Coddington, J. A. & Levi, H. W. *Annu. Rev. Ecol. Syst.* **22**, 565–592 (1991).
- Damen, W. G. M., Saridaki, T. & Averof, M. *Curr. Biol.* **12**, 1711–1716 (2002).
- Guerette, P. A., Ginzinger, D. G., Weber, B. H. F. & Gosline, J. M. *Science* **272**, 112–115 (1996).
- Gatesy, J., Hayashi, C., Motriuk, D., Woods, J. & Lewis, R. *Science* **291**, 2603–2605 (2001).
- Garb, J. E., DiMauro, T., Vo, V. & Hayashi, C. Y. *Science* **312**, 1762 (2006).

Supplementary information accompanies this communication on Nature's website.

Received 3 July; accepted 25 August 2006.

Competing financial interests: declared none.
doi:10.1038/443407a

BRIEF COMMUNICATIONS ARISING online
www.nature.com/bca see Nature contents.

S. NIEDEREGGER

PLANT GENETICS

Increased outcrossing in *hothead* mutantsArising from: S. J. Lolle, J. L. Victor, J. M. Young & R. E. Pruitt *Nature* **434**, 505–509 (2005)

Lolle *et al.*¹ report that loss-of-function alleles of the *HOTHEAD* (*HTH*) gene in *Arabidopsis thaliana* are genetically unstable, giving rise to wild-type revertants. On the basis of the reversion of many other genetic markers in *hth* plants, they suggested a model in which a cache of extragenomic information could cause genes to revert to the genotype of previous generations. In our attempts to reproduce this phenomenon, we discovered that *hth* mutants show a marked tendency to outcross (unlike wild-type *A. thaliana*, which is almost exclusively self-fertilizing²). Moreover, when *hth* plants are grown in isolation, their genetic inheritance is completely stable. These results may provide an alternative explanation for the genome wide non-mendelian inheritance reported by Lolle *et al.*

Initially, we constructed *hth-12 gl1-4* double-mutant plants in the Columbia ecotype, reasoning that *HTH* and *GL1* should revert independently because they are on different chromosomes. *hth-12* DNA carries a transfer-DNA (T-DNA) insertion (SALK_024611) and *gl1-4* is a guanine-to-adenine (G-to-A) transition mutation (like that shown previously to revert¹) that changes the start codon of the trichome gene *GL1* (ref. 3) from ATG to ATA. Among 1,597 progeny of *hth-12 gl1-4* plants, 10 were phenotypically *GL1* (normal trichomes). Genotyping based on polymerase chain reaction showed that nine were heterozygous for *gl1-4*, and one was *GL1/GL1*. Surprisingly, the nine *GL1/gl1-4* plants were also heterozygous for *hth-12*, and the *GL1/GL1* homozygote was homozygous for *HTH*. These observations are most easily explained by pollen contamination (nine heterozygous plants) and seed contamination (one homozygous plant). We also found a single *hth-12* heterozygote that

was still homozygous for *gl1-4*, which could be explained by pollen contamination from nearby *gl1-4* plants.

To test whether pollen contamination could be a source of apparent *hth* genetic reversion, we grew homozygous *hth-12* plants either in a mixed population (near to, but not touching, plants with varied genotypes) or in an isolated room containing only *hth-12* plants. In one experiment, the progeny of plants grown in the mixed-growth room showed 19/245 revertants (Table 1). Eighteen of nineteen revertants segregated the *erecta* phenotype in the next generation, suggesting that they arose from pollen contamination by nearby *erecta*-containing plants.

In a second mixed-population experiment, 18/415 plants were phenotypically *HTH*. All 18 contained a *BIN2-1::GFP* transgene⁴, which was present in other plants grown in the room (Table 1). In contrast, not a single revertant was found among 932 progeny of *hth-12* plants grown in isolation.

We repeated these experiments with the originally reported *hth-8* and *hth-5* alleles in the Landsberg *erecta* (*Ler*) ecotype^{1,5}. We found that *hth-8* plants grown in mixed populations yielded 156/994 progeny with a *HTH* phenotype. Most were either *ERECTA* or contained *BIN2-1::GFP* (Table 1). However, *hth-8* plants grown in isolation gave exclusively *hth* progeny, none of which was *ERECTA* (Table 1). Similar results were obtained with *hth-5*.

Our results indicate that *hth* mutants are particularly susceptible to pollen contamination, possibly because the *hth* floral organ fusion defects lead to inefficient self-pollination and exerted stigmas¹, or because of changes in cuticle composition⁵. This tendency to outcross may provide an alternative

Table 1 | Outcrossing in *hth* mutants

Genotype	Number of phenotypically revertant plants	
	Mixed population	Isolated population
<i>hth-12</i>	19/245 (7.8%)*	0/295 (0%)
<i>hth-12</i>	18/415 (4.3%)*†	0/637 (0%)
<i>hth-8</i>	156/994 (15.7%)*‡	0/890 (0%)*§
<i>hth-5</i>	22/1144 (1.9%)*	0/913 (0%)*§

Homozygous *hth* plants were grown in a room with plants of mixed genotype (mixed population) or in isolation (isolated population). Progeny from these two populations were scored for plants with the wild-type *HTH* phenotype. (Plants were cared for by Yu Li, Shawn Cokus, Lynn Jacobsen, Zhongliang Peng and Suwen Wang. *BIN2-1::GFP* seeds were provided by Jianming Li.)

*Of 19 phenotypically *HTH* plants, 18 segregated *erecta* in the next generation.

†All 18 revertants segregated a *BIN2-1::GFP* transgene in the next generation.

‡Among 156 *HTH* revertants, 145 were *ERECTA*, four were dwarf plants containing the *BIN2-1::GFP* transgene, and seven seemed similar to *Ler* plants.

§All plants also retained an *erecta* phenotype.

||All 22 revertants were also *ERECTA*.

explanation for the apparent genetic instability of *hothead* mutants.

Peng Peng*†, Simon W.-L. Chan†‡, Govind A. Shah†, Steve E. Jacobsen*†

*Howard Hughes Medical Institute, †Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, California 90095, USA

e-mail: jacobsen@ucla.edu

‡Present address: Section of Plant Biology, University of California, Davis, California 95616, USA

- Lolle, S. J., Victor, J. L., Young, J. M. & Pruitt, R. E. *Nature* **434**, 505–509 (2005).
- Abbott, R. J. & Gomes, M. F. *Heredity* **62**, 411–418 (1989).
- Herman, P. L. & Marks, M. D. *Plant Cell* **1**, 1051–1055 (1989).
- Li, J. & Nam, K. H. *Science* **295**, 1299–1301 (2002).
- Krolikowski, K. A., Victor, J. L., Wagler, T. N., Lolle, S. J. & Pruitt, R. E. *Plant J.* **35**, 501–511 (2003).

doi:10.1038/nature05251

PLANT GENETICS

Lolle *et al.* reply

Replying to: P. Peng, S. W.-L. Chan, G. A. Shah & S. E. Jacobsen *Nature* **443**, doi:10.1038/nature05251 (2006).

The results obtained by Peng *et al.*¹ are consistent with an increased amount of outcrossing in *hth* mutants of *Arabidopsis thaliana*. Some of the results, such as the acquisition of a novel transgene, would be difficult to explain by any other mechanism. Outcrossing was a possibility that we thoroughly explored early on in our investigation², but we discounted it as an explanation because it was inconsistent with

many of our experimental results.

We described two experiments that were inconsistent with outcrossing²: one in which there was transmission of a wild-type *HTH* allele from a homozygous mutant (*hth/hth*) male parent, and another in which there was recovery of homozygous wild-type (*HTH/HTH*) embryos dissected from homozygous mutant (*hth/hth*) parents. In further experi-

ments that were similar, but not identical, to those described by Peng *et al.*¹, we did see reversion in *hth/hth* homozygotes grown in isolation (results not shown).

These results together indicate that the genetic events that we see in *hth* mutants cannot be explained solely by outcrossing, but they do not rule out the possibility that outcrossing could be increased relative to that occurring in the wild type. The lower frequency of reversion seen when pollen from an *hth/hth* parent is used to pollinate a wild-type female may reflect elimination of these outcrossing events.

We have also examined more extensive patterns of inheritance of single-nucleotide

polymorphisms in F_2 populations, similar to those we originally described². These patterns of inheritance are also inconsistent with an outcrossing explanation because there was no single male parent present that could have provided the combination of non-parental alleles observed in the 'restored' progeny. Furthermore, the results indicate that genetic restoration of ancestral alleles can take place in *HTH/hth* heterozygotes; these plants have a floral morphology identical to wild type and therefore would not be expected to show increased outcrossing (J.M.Y., R.E.P.

and S.J.L., unpublished results).

In summary, the outcrossing explanation proposed by Peng *et al.*¹ is a reasonable hypothesis to explain some of the data associated with *hothead* genetics, and indeed is one of the first that we considered. Ultimately, we discarded this explanation because it was inconsistent with many of our experimental results. However, the results of Peng *et al.* show that, at least under some growth conditions, outcrossing in *hth/hth* plants remains an issue that needs to be taken into account.

Susan J. Lolle*, **Robert E. Pruitt†**,

J. L. Victor†, **J. M. Young†**

*Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

†Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907-2054, USA

e-mail: pruittr@purdue.edu

1. Peng, P., Chan, S. W.-L., Shah, G. A. & Jacobsen, S. E. *Nature* **443**, doi:10.1038/nature05251 (2006).
2. Lolle, S. J., Victor, J. L., Young, J. M. & Pruitt, R. E. *Nature* **434**, 505–509 (2005).

doi:10.1038/nature05252

Bose–Einstein condensation of exciton polaritons

J. Kasprzak¹, M. Richard², S. Kundermann², A. Baas², P. Jeambrun², J. M. J. Keeling³, F. M. Marchetti⁴, M. H. Szymańska⁵, R. André¹, J. L. Staehli², V. Savona², P. B. Littlewood⁴, B. Deveaud² & Le Si Dang¹

Phase transitions to quantum condensed phases—such as Bose–Einstein condensation (BEC), superfluidity, and superconductivity—have long fascinated scientists, as they bring pure quantum effects to a macroscopic scale. BEC has, for example, famously been demonstrated in dilute atom gas of rubidium atoms at temperatures below 200 nanokelvin. Much effort has been devoted to finding a solid-state system in which BEC can take place. Promising candidate systems are semiconductor microcavities, in which photons are confined and strongly coupled to electronic excitations, leading to the creation of exciton polaritons. These bosonic quasi-particles are 10^9 times lighter than rubidium atoms, thus theoretically permitting BEC to occur at standard cryogenic temperatures. Here we detail a comprehensive set of experiments giving compelling evidence for BEC of polaritons. Above a critical density, we observe massive occupation of the ground state developing from a polariton gas at thermal equilibrium at 19 K, an increase of temporal coherence, and the build-up of long-range spatial coherence and linear polarization, all of which indicate the spontaneous onset of a macroscopic quantum phase.

Bosons—particles with an integer spin—can undergo BEC when their de Broglie wavelength becomes comparable to their average separation. Then a large fraction of the bosons condense in the lowest quantum state, resulting in the appearance of macroscopic coherence. Massive occupation of the ground state^{1,2} and the expected spontaneous coherence^{3–5} have been clearly demonstrated for dilute atom gases cooled down to temperatures of about 10^{-6} K.

For solid-state systems, excitons in semiconductors have long been considered a promising candidate for BEC at temperatures of a few kelvin, reachable by standard cryogenic techniques^{6–8}. Excitons are light-mass Bose particles analogous to positronium, consisting of bound electron–hole pairs, usually produced by optical excitation. Over the past three decades there have been numerous studies and early claims of exciton BEC in three-dimensional (3D) semiconductors (for a recent review see refs 9 and 10). Other systems in the solid state have also exhibited unusual physical properties tentatively attributed to BEC^{11,12}.

Recently new directions have been explored for exciton BEC, using two-dimensional (2D) quantum structures, such as coupled quantum wells under an external applied electric field^{13,14}, or quantum wells embedded in optical microcavities. The second system consists in planar Fabry–Perot resonators whose optical length is tuned to a half-integer multiple of the emission wavelength of quantum well excitons. The near-degeneracy and strong coupling of the exciton and cavity photon leads to the formation of new eigenstates (see Fig. 1) called polaritons, which are half-light, half-matter bosonic quasi-particles^{15,16}. The extremely steep dispersion of the cavity polariton modes, due to the optical confinement along the z direction, results in a typical polariton effective mass of 10^{-4} times the free electron mass. Thus, in theory, the temperature and density criteria for BEC of polaritons in their $k_{\parallel} = 0$ ground state should be satisfied much more easily than for excitons. Moreover, from the experimental side,

we note that the coherence, polarization and population distribution properties of polaritons can be conveniently probed by analysing the far-field emission, because the light emitted by the microcavity is part of the polariton wavefunction¹⁷. The drawback of the strong coupling is the very short polariton lifetime, typically of around 10^{-12} s, which could be an obstacle to reach thermal equilibrium. Although full thermalization cannot be achieved with the host lattice, it will be shown below that polariton–polariton scattering processes are fast enough under high excitation to produce a fully thermalized polariton gas.

The first indication of spontaneous quantum degeneracy of polaritons was the observation of stimulated emission under non-resonant pumping in CdTe microcavities¹⁸: above some excitation power threshold, the polariton emission exhibits a strong nonlinearity, while the linewidth shows significant narrowing. Since then several claims of polariton condensation have been made. In particular, the second-order time correlations in a GaAs-based microcavity were measured to support such a claim¹⁹. However, no measurement of the polarization or of the spatial coherence is performed. Also, the second-order correlations do not follow the expected signature: at the stimulation threshold they are found to be quasi-thermal, and above threshold remain far from that expected for a coherent state. Of the defining features of BEC—that is, spontaneous symmetry breaking and long-range order^{20,21}—no direct proof in semiconductor systems has ever been given^{9,10}.

Of course, an infinite 2D system never develops true long-range order (see the Supplementary Information)²². However, owing to the finite size of the excitation spot, the size of the polariton cloud is finite, and one can achieve complete coherence across the cloud at low enough temperatures and macroscopic occupation of a single quantum state. Polaritons also have some specific features: first, they exhibit strong interactions even at modest densities^{23,24}, so the

¹CEA-CNRS-UJF joint group 'Nanophysique et Semiconducteurs', Laboratoire de Spectrométrie Physique (CNRS UMR5588), Université J. Fourier-Grenoble, F-38402 Saint Martin d'Hères cedex, France. ²Ecole Polytechnique Fédérale de Lausanne (EPFL), Station 3, CH-1015 Lausanne, Switzerland. ³MIT, Department of Physics, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139-4307, USA. ⁴Cavendish Laboratory, Department of Physics, University of Cambridge, J. J. Thomson Avenue, Cambridge CB3 0HE, UK.

⁵Clarendon Laboratory, Department of Physics, University of Oxford, Parks Road, Oxford OX1 3PU, UK.

physics soon exits the regime of weakly interacting bosons that describes ultracold atoms; second, the lifetime is short enough that we must confront the role of non-equilibrium physics²⁵. Nevertheless, the principal experimental characteristics expected for BEC are clearly reported here: condensation into the ground state arising out of a population at thermal equilibrium; the development of quantum coherence, indicated by long-range spatial coherence, and sharpening of the temporal coherence of the emission.

Experimental procedure

The sample we studied consists of a CdTe/CdMgTe microcavity grown by molecular beam epitaxy. It contains 16 quantum wells,

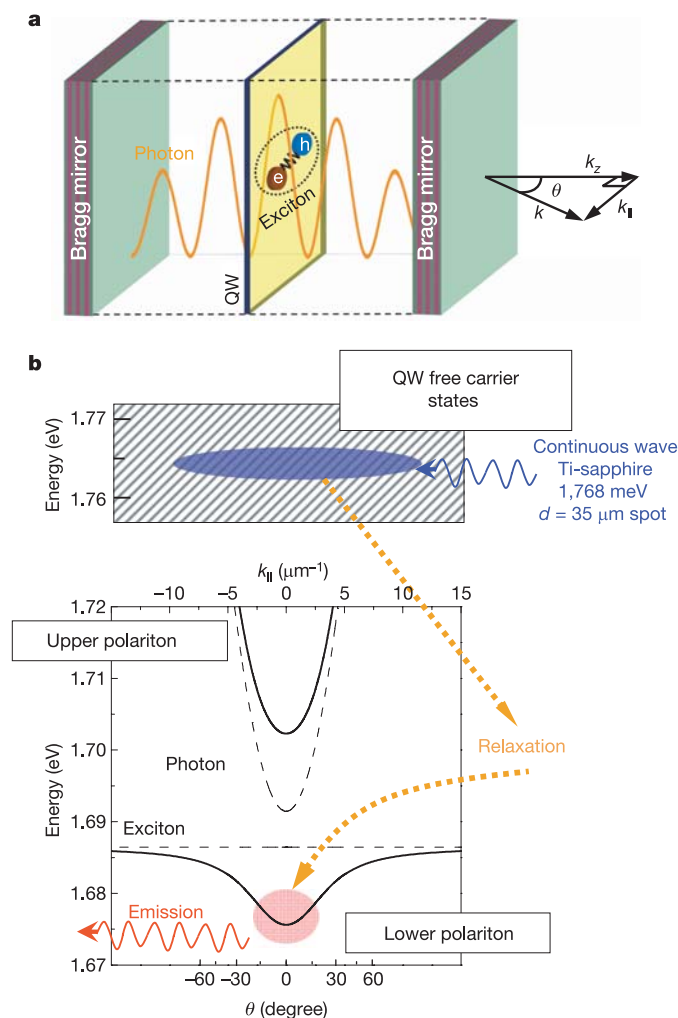


Figure 1 | Microcavity diagram and energy dispersion. **a**, A microcavity is a planar Fabry–Perot resonator with two Bragg mirrors at resonance with excitons in quantum wells (QW). The exciton is an optically active dipole that results from the Coulomb interaction between an electron in the conduction band and a hole in the valence band. In microcavities operating in the strong coupling regime of the light–matter interaction, 2D excitons and 2D optical modes give rise to new eigenmodes, called microcavity polaritons. **b**, Energy levels as a function of the in-plane wavevector $k_{\parallel}$ in a CdTe-based microcavity. Interaction between exciton and photon modes, with parabolic dispersions (dashed curves), gives rise to lower and upper polariton branches (solid curves) with dispersions featuring an anticrossing typical of the strong coupling regime. The excitation laser is at high energy and excites free carrier states of the quantum well. Relaxation towards the exciton level and the bottom of the lower polariton branch occurs by acoustic and optical phonon interaction and polariton scattering. The radiative recombination of polaritons results in the emission of photons that can be used to probe their properties. Photons emitted at angle θ correspond to polaritons of energy E and in-plane wavevector $k_{\parallel} = (E/\hbar c)\sin\theta$.

displaying a vacuum field Rabi splitting of 26 meV (ref. 26). The microcavity was excited by a continuous-wave Ti:sapphire laser, combined with an acousto-optic modulator (1- μ s pulse, 1% duty cycle) to reduce sample heating. The pulse duration is sufficiently long (by four orders of magnitude) in comparison with the characteristic times of the system to guarantee a steady-state regime. The laser beam was carefully shaped into a ‘top hat’ intensity profile providing a uniform excitation spot of about 35 μ m in diameter on the sample surface, as shown in Fig. 4i. The excitation energy was 1.768 eV, well above the polariton ground state (1.671 eV at cavity exciton resonance), at the first reflectivity minimum of the Bragg mirrors, allowing proper coupling to the intra-cavity field. This ensures that polaritons initially injected in the system are incoherent, which is a necessary condition for demonstrating BEC. In atomic BEC or superfluid helium, the temperature is the parameter driving the phase transition. Here the excitation power, and thus the injected polariton density, is an easily tunable parameter, and so we chose it as the experimental control parameter. The large exciton binding energy in CdTe quantum wells (25 meV), combined with the large number of quantum wells in the microcavity, is crucial in maintaining the strong coupling regime of polaritons at high carrier density. The far-field polariton emission pattern was measured to probe the population distribution along the lower polariton branch. The spatially resolved emission and its coherence properties are accessible in a real-space imaging set-up combined with an actively stabilized

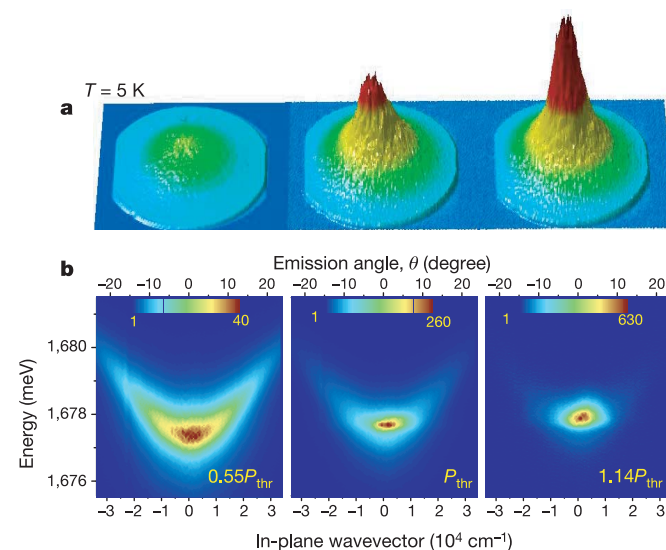


Figure 2 | Far-field emission measured at 5 K for three excitation intensities. Left panels, $0.55 P_{\text{thr}}$; centre panels, P_{thr} and right panels, $1.14 P_{\text{thr}}$; where $P_{\text{thr}} = 1.67 \text{ kW cm}^{-2}$ is the threshold power of condensation. **a**, Pseudo-3D images of the far-field emission within the angular cone of $\pm 23^\circ$, with the emission intensity displayed on the vertical axis (in arbitrary units). With increasing excitation power, a sharp and intense peak is formed in the centre of the emission distribution ($\theta_x = \theta_y = 0^\circ$), corresponding to the lowest momentum state $k_{\parallel} = 0$. **b**, Same data as in **a** but resolved in energy. For such a measurement, a slice of the far-field emission corresponding to $\theta_x = 0^\circ$ is dispersed by a spectrometer and imaged on a charge-coupled device (CCD) camera. The horizontal axes display the emission angle (top axis) and the in-plane momentum (bottom axis); the vertical axis displays the emission energy in a false-colour scale (different for each panel; the units for the colour scale are number of counts on the CCD camera, normalized to the integration time and optical density filters, divided by 1,000 so that 1 corresponds to the level of dark counts: 1,000). Below threshold (left panel), the emission is broadly distributed in momentum and energy. Above threshold, the emission comes almost exclusively from the $k_{\parallel} = 0$ lowest energy state (right panel). A small blue shift of about 0.5 meV, or 2% of the Rabi splitting, is observed for the ground state, which indicates that the microcavity is still in the strong coupling regime.

Michelson interferometer to study phase spatial correlations. Experiments discussed in this work were performed for a slightly positive cavity–exciton detuning (3 to 8 meV).

Thermalization and condensation

Under non-resonant and high excitation, the polariton emission in CdTe-based microcavities becomes highly nonlinear^{18,27–30}. We first analyse the spectral and angular distribution of the emission as a function of the excitation power. Figure 2a displays pseudo-3D images of the angular distribution of the spectrally integrated emission. Below threshold (left), the emission exhibits a smooth distribution around $\theta_x = \theta_y = 0^\circ$, that is, around $k_{\parallel} = 0$. When the excitation intensity is increased, the emission from the zero momentum state becomes predominant at threshold (centre) and a sharp peak forms at $k_{\parallel} = 0$ above threshold (right). Figure 2b shows the energy and angle-resolved emission intensities. The width of the momentum distribution shrinks with increasing excitation intensity, and above threshold, the emission mainly comes from the lowest energy state at $k_{\parallel} = 0$. The polariton occupancy has been extracted from such emission patterns by taking into account the radiative lifetime of polaritons.

Figure 3a shows the occupancy of the ground state, as well as its emission energy and linewidth as a function of excitation power. With increasing excitation power, the occupancy first increases linearly, then exponentially, with sharp threshold-like behaviour. It should be noted that the occupancy at threshold is close to unity, consistent with a polariton relaxation process stimulated by the ground-state population: a specific feature of bosons. The emission blue shift was measured to be less than a tenth of the Rabi splitting at a pumping level ten times above the nonlinear threshold, confirming that the microcavity is still in the strong coupling regime. We measured the ordinary lasing excitation threshold and found it to be 50 times higher than the condensation threshold (not shown).

The linewidth of the $k_{\parallel} = 0$ emission shows significant narrowing

at the nonlinear threshold^{29,30}, down to half of the polariton linewidth in the linear regime (Fig. 3a). The line broadening observed at higher excitation is due to decoherence induced by polariton self-interaction³¹. We studied the coherence time more directly using a Michelson interferometer (not shown). This measurement gives a coherence time of 1.5 ps below the nonlinear threshold, and 6 ps above threshold, consistent with the spectral narrowing observed at threshold.

Signatures of polariton coherence in CdTe-based microcavities have been previously reported^{28,29}. Macroscopic coherence in the momentum plane was observed above the nonlinear threshold²⁸. However, the use of a small excitation spot (3 μm diameter) prevented relaxation into the lowest polariton energy states: polariton stimulation occurred in excited states and was thus only remotely connected with BEC. An experiment under conditions more favourable to BEC (25- μm -diameter spot), in which polaritons could condense into the lowest energy state, indirectly showed the build-up of macroscopic coherence in real space above threshold²⁹. However, that measurement was obtained under pulsed excitation (150-fs pulses), thus precluding steady state in the system and mixing high polariton densities at short times and low densities at long times on the same spectra.

Figure 3b displays the occupancy of polaritons as a function of their energy. The occupancy is computed by measuring the intensity of the signal, taking into account the polariton radiative recombination rate and the efficiency of the collection set-up. The uncertainty may be estimated to be roughly a factor of two. The estimation has been performed for different detunings and the threshold is always observed for occupancies of the order of one, in agreement with all previous measurements. For the sake of simplicity, we have arbitrarily adjusted the ground-state occupancy to be one at threshold. At very low excitation power, the polariton occupancy is not thermalized^{29,32,33}. Close to threshold, the occupancy can be fitted with a Maxwell–Boltzmann distribution, indicating a polariton gas in

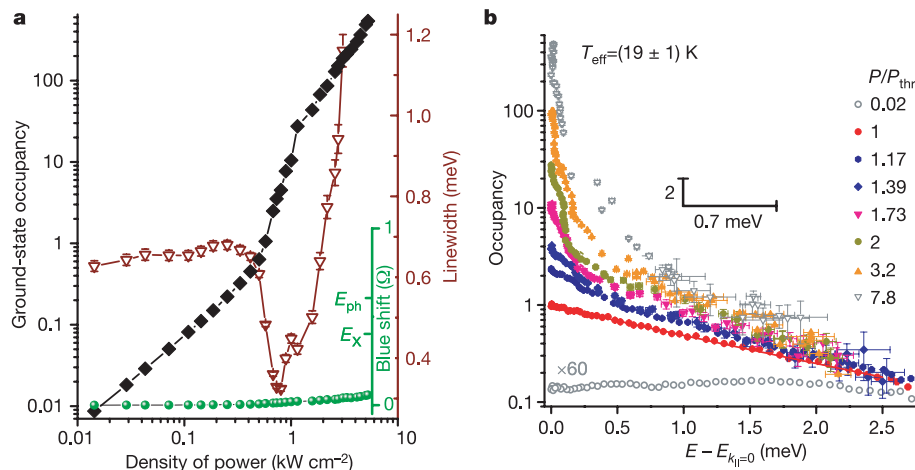


Figure 3 | Polariton occupancy measured at 5 K. **a**, Occupancy of the $k_{\parallel} = 0$ ground state (solid black diamonds), its energy blue shift (solid green circles) and linewidth (open red triangles) versus the excitation power. The blue shift is plotted in units of the Rabi splitting $\Omega = 26$ meV. At low excitation densities, the ground-state occupancy increases linearly with the excitation and then, immediately after threshold, increases exponentially before becoming linear again. This sharp transition is accompanied by a decrease of the linewidth by about a factor of two, corresponding to an increase of the polariton coherence. Further increase in linewidth is due to interaction between polaritons in the condensate. The polariton ground state slightly blue shifts, by less than 7% of the Rabi splitting for densities up to seven times the threshold density, staying well below the uncoupled exciton (E_X) and photon mode (E_{ph}) energies. This provides clear evidence of the strong coupling regime. **b**, Polariton occupancy in ground- and excited-state levels is plotted in a semi-logarithmic scale for various

excitation powers. For each excitation power, the zero of the energy scale corresponds to the energy of the $k_{\parallel} = 0$ ground state. The occupancy is deduced from far-field emission data (see Fig. 2b), taking into account the radiative lifetime of polaritons. At the excitation threshold, the polariton gas is fully thermalized, as indicated by the Boltzmann-like exponential decay of the distribution function. Above threshold, the ground state becomes massively occupied, whereas the excited states are saturated, which is typical of BEC. The polariton thermal cloud is found to be at 19 K without significant changes when increasing the excitation to twice the threshold power. The low-energy part of the polariton occupancy cannot usually be properly fitted by a Bose distribution function, as expected for BEC of interacting particles. The error bars indicate standard deviation for each point; and the absolute uncertainty in occupation factor and polariton energy is given as the black scale bars.

thermal equilibrium at around 19 K. Above threshold, one can clearly observe a distribution featured by the saturation of the excited-state population and the formation of a condensate in the ground state. Saturation of excited levels and stability of the polariton temperature persist within a range $0.9 < P/P_{\text{thr}} < 2.3$ of excitation power. Such behaviour is typical of BEC, but, in general, we cannot obtain a reasonable fit for the low-energy part of the occupancy with a Bose–Einstein distribution. This is because in a non-ideal Bose system, interactions are responsible for changes in the density of states and depletion of the condensate in favour of excited states; such effects have been seen in atomic- and liquid-helium experiments^{34,35}. At first sight, the observation of an internal thermal equilibrium for polaritons could appear puzzling, considering the short polariton radiative lifetime. In fact, strong broadening of the polariton emission along the dispersion curve for excitation around threshold shows that the dephasing time due to polariton–polariton scattering is shorter than the polariton lifetime by a factor of two (not shown). Such a mechanism has been proposed for polariton BEC³⁶ and could indeed permit attainment of internal thermal equilibrium before the escape of polaritons out of the microcavity. Above threshold, the relaxation towards the condensed state should be enhanced thanks to stimulated scattering, whereas the polaritons are not expected to exhibit a reduced lifetime at high density. This fact should even favour the attainment of thermodynamical equilibrium in the condensed phase.

It is instructive to compare the density and temperature of thermalized polaritons (extracted from the occupancy of Fig. 3) with the theoretical predictions of the phase boundary^{23,24}. At $P = 0.9 P_{\text{thr}}$, where thermalization but no condensation is observed, we estimate a density of around $5 \times 10^8 \text{ cm}^{-2}$, which lies just above the theoretical threshold density for condensation at $T_{\text{eff}} = 19 \text{ K}$ (see Supplementary Information).

Because BEC is a transition from an incoherent population to a

coherent matter wave, the most important property to be explored is the order parameter of the system, the macroscopic wavefunction of the condensate. As stated before, the condensed fraction should be described by a single wavefunction, and so we expect a clear polarization of the light, and a stationary phase across the whole condensate.

Linear polarization build-up

We measured the linear and circular polarization of the polariton emission, using either circularly or linearly polarized excitation. Below the nonlinear threshold, the emission appears to be completely depolarized (Fig. 4a–c). However, above threshold, a linear polarization of up to 83% spontaneously develops for $k_{\parallel} = 0$ polaritons, whatever the polarization of the excitation (Fig. 4a, d, e)³⁷. The polarization direction is found to be approximately aligned along the [110] axis of the microcavity. For this striking result we consider possible explanations other than BEC.

First, any Bragg-based microcavity displays a longitudinal/transverse splitting due to the Fresnel laws. However, this splitting should vanish for vanishing incidence angle, that is for $k_{\parallel} = 0$. Second, it could be argued that this polarization is inherited from the excitation through some parametric amplification process^{38,39}. Then, in this case, the polarization of the emission above the nonlinear threshold should be strongly dependent on the laser polarization. Such a correlation was not observed for either circularly or linearly polarized excitation (Fig. 4a), which rules out the hypothesis of parametric amplification.

We also checked the polarization of the emission spot. Below threshold the emission spot is homogeneous (Fig. 4h) and depolarized (not shown). Above threshold it becomes highly inhomogeneous, breaking into several $\sim 4\text{-}\mu\text{m}$ spots which emit with the same linear polarization (see Fig. 4f and g). A careful examination of

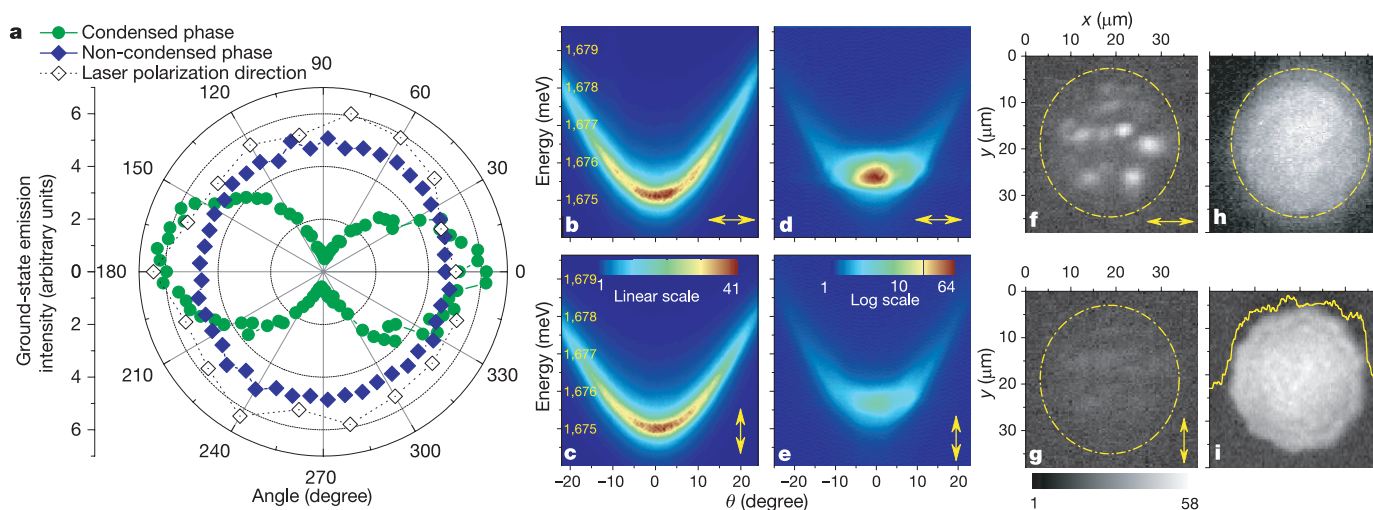


Figure 4 | Polarization properties of the polariton emission. **a**, The polar plot displays the intensity of the ground state emission at $k_{\parallel} = 0$ (within a 0.4° aperture) measured as a function of the angle of the linear analyser. Below threshold (solid blue diamonds), the emission is completely depolarized for linear and circular (not shown) polarization, whereas above threshold (solid green circles) a linear polarization exceeding 80% is observed. The linear polarization is 'horizontal' (0° , 180°), roughly aligned along the [110] crystalline axis. Open diamonds represent the intensity of the linearly polarized emission above threshold, measured as a function of the linear polarization angle of the excitation laser. No correlation can be found between the excitation polarization and the polariton polarization. **b–e**, Analysis of the linear polarization (the doubleheaded yellow arrows indicate vertical or horizontal polarization of the detection) of the polariton emission below (**b**, **c**) and above (**d**, **e**) threshold. Below threshold, polaritons along the dispersion curve are not polarized because their

emission intensity is the same for horizontal and vertical polarizations. Above threshold, emission from the excited states remains depolarized, but emission from the ground state is strongly linearly polarized. Note the linear and logarithmic scales (number of counts on the CCD divided by 1,000) used for the emission intensity measured below and above threshold, respectively. **b**, **d** and **e** have the same axes as **c**. **f–i**, Analysis of the linear polarization of the emitting spot below (**h**) and above (**f**, **g**) threshold. **i** shows the image of the excitation laser with its 'top hat' intensity profile. The greyscale applies to **f** and **g** (number of counts on the CCD camera divided by 1,000). **h** shows the emitting spot below threshold which appears homogeneous and depolarized (not shown). Above threshold, the emitting spot becomes inhomogeneous: several bright small spots, due to structural inhomogeneities, are observed within the excitation area. Each of them emits with the same linear polarization, consistent with the development of a single condensate. **h** and **i** have the same axes as **f** and **g**.

the emission below threshold reveals a splitting of the order of 0.1 meV between two linearly cross-polarized emissions at $k_{\parallel} = 0$, most probably due to anisotropic photonic structural disorder. This splitting is much smaller than $kT \approx 1.6$ meV, and yet above threshold, the system selects the lowest energy of these two states⁴⁰. Such a selection, as observed in atomic BEC, is a strong indication that a phase transition has occurred.

The final step of our demonstration is the evidence of macroscopic phase coherence^{20,21}, that is, the direct measurement of long-range spatial correlations. In particular, it will rule out the possibility of several independent condensates.

Long-range spatial coherence

Spatial coherence has been investigated by measuring the classical first-order correlation function of the polariton emission:

$$g^{(1)}(\mathbf{r}, \mathbf{r}') = \frac{\langle E^*(\mathbf{r})E(\mathbf{r}') \rangle}{\langle E^*(\mathbf{r}) \rangle \langle E(\mathbf{r}') \rangle}$$

where $E(\mathbf{r})$ is the electric field at point $\mathbf{r}$. For classical fields, $g^{(1)}(\mathbf{r}, \mathbf{r}')$ gives the amount of phase correlations between the fields at points $\mathbf{r}$ and $\mathbf{r}'$ without relative time delay. In the low-density regime, the polaritons are expected to exhibit short-range correlations, with a correlation length given by the thermal de Broglie wavelength. In the condensed phase, complete coherence, up to the size of the condensate is expected^{20,21}.

We combined a Michelson interferometer with a high-resolution

imaging set-up: two images of the condensate magnified 40 times, each coming from one arm of the interferometer, are combined at the output of the interferometer and overlap in the image plane, forming an interference pattern. One image can be displaced with respect to the other by any vector $\mathbf{d}$ simply by tilting a mirror of the interferometer. The contrast C of the interference is measured for each point of the image plane by scanning the relative phase of the interferometer over $\sim 6\pi$, providing a direct measurement of the correlation function:

$$C(\mathbf{r}, \mathbf{d}) = \frac{I_{\max} - I_{\min}}{I_{\max} + I_{\min}} = \frac{2\sqrt{I(\mathbf{r})I(\mathbf{r}+\mathbf{d})}}{I(\mathbf{r}) + I(\mathbf{r}+\mathbf{d})} g^{(1)}(\mathbf{r}, \mathbf{r}+\mathbf{d})$$

in which I denotes light intensity and $I_{\max}$ and $I_{\min}$ denote the maximum and the minimum of the interference pattern (intensity versus phase). We first measured the first-order correlation between two small regions ($\sim 4 \mu\text{m}^2$) of the emission separated by $6 \mu\text{m}$ (see Fig. 5a, b), as a function of the driving parameter—the excitation power. Below threshold, the interference contrast is below 5% resolution limited. Above the stimulation threshold, the contrast grows up to 45%, indicating a significant increase in the correlation length. Similar results have been obtained for any pair of bright spots chosen within the condensate.

Next, we measured the correlations between any pair of points $\mathbf{r}$ and $\mathbf{r}'$ symmetric with respect to the centre of the condensate. To do so, we replaced the mirror in one arm of the interferometer by a retro-reflector to invert the image in a centro-symmetric way. Thus,

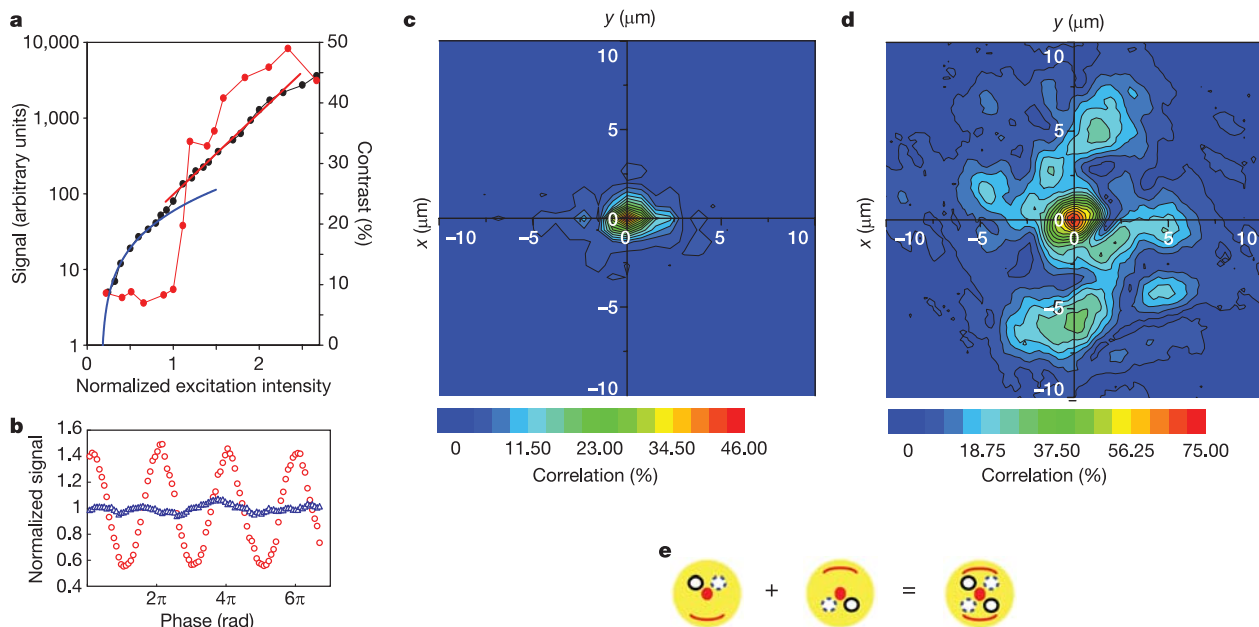


Figure 5 | Spatial correlation measurements using a Michelson interferometer. **a**, Solid red circles indicate correlations between two spots separated by $6 \mu\text{m}$ (2.5 times the thermal de Broglie wavelength) within the condensate as a function of the excitation power (the threshold power was $P_{\text{thr}} = 4.5 \text{ kW cm}^{-2}$). The correlation exhibits a threshold-like behaviour: it starts to build up from a noise-limited value of 5–8% to 46% at excitation power twice the threshold power. The variation of the ground-state emission intensity, normalized to the excitation power, is shown for comparison (solid black circles). The solid blue line is a quadratic fit of the data demonstrating the occurrence of particle–particle interaction below threshold. Above threshold, the solid red line is an exponential fit demonstrating the strong stimulation of the relaxation by the high occupancy factor of the ground state. **b**, Typical interference fringes between spots 1 and 2 as a function of the relative phase (horizontal axis), measured below (open blue triangles) and above (open red circles) the nonlinear threshold, with contrasts of 5% and 46%, respectively. **c**, Correlation mapping below threshold using a linear colour scale. Each point (x, y) in the

map represents the correlation between points (x, y) and $(-x, -y)$ of the condensate (see **e** for details). The correlation peak extends over $2.6 \mu\text{m}$ (full-width at half-maximum), thus providing a measurement of the de Broglie wavelength. **d**, Same as **c** but for excitation above threshold. Some islands with a high degree of correlation (up to 30%) are formed for distances as long as 4.5 times the thermal de Broglie wavelength. These islands correspond to the bright spots caused by the in-plane spatial disorder experienced by the condensate (see Fig. 4f). **e**, Schematic description of the experiment realized for correlation mapping in **c** and **d**. The first ‘smiley’ symbolizes the image originating from the first arm of the Michelson interferometer. The second arm produces an identical but flipped image with respect to a chosen point (here, the red nose). The resulting interference pattern consists in the overlap of the two images, each point corresponding to the interference between one point of the ‘smiley’ and its symmetric with respect to the nose. All experiments were done using a $20\text{-}\mu\text{m}$ -diameter spot with a gaussian intensity profile.

the contrast of the interference pattern gives $g^{(1)}(\mathbf{r}, -\mathbf{r})$. This configuration simultaneously provides the correlation between pairs of points separated by the quantity $\mathbf{d} = -2\mathbf{r}$ (Fig. 5e).

Correlations measured for $P = 1.9P_{\text{thr}}$ are displayed in Fig. 5d. Apart from the high correlation peak at the centre, which corresponds to the short-range correlations, several large islands with correlations exceeding 30% are observed for pairs of points separated by $|\mathbf{d}| = 12\ \mu\text{m}$. The range of these correlations is limited only by the finite size of the condensate. The variety of the island shapes reflects only the spatial inhomogeneity inherited from the in-plane disorder of the microcavity.

Below the nonlinear threshold, we find that the correlations vanish for distances as small as $2.6\ \mu\text{m}$. This coherence length corresponds to the thermal de Broglie wavelength of a homogenous thermalized polariton gas (see Fig. 3b and Fig. 4h). The condensate cloud is several times larger than this length ($16\ \mu\text{m}$ versus $2.6\ \mu\text{m}$), yet shows a correlations length of the order of its diameter, providing direct evidence of coherence across the entire condensate.

In conclusion, we have realized the condensation of exciton polaritons in a CdTe-based microcavity. Strictly speaking, the reported condensation is not a standard BEC because polaritons are not ideal (non-interacting) bosons, and are not in thermal equilibrium with the phonon bath. Moreover the polariton system is not conservative, in the sense that polaritons continuously leak out of the microcavity. Nevertheless all specific ingredients of a true BEC have been observed: above some critical density, condensation takes place in the ground state, out of a degenerate Bose gas fully thermalized at 19 K. The phase-transition character of the phenomenon is clearly seen, to our knowledge for the first time in the solid state, by the build-up of spatial coherence and macroscopic polarization across the entire condensate. These findings are promising for the development of the so-called 'polariton laser'^{16,41} and Bose condensation at increased temperatures with wider bandgap semiconductors such as ZnO or GaN.

Received 18 April; accepted 24 July 2006.

- Anderson, M. N. *et al.* Observation of Bose-Einstein condensation in a dilute atomic vapor. *Science* **269**, 198–201 (1995).
- Davis, K. B. *et al.* Bose-Einstein condensation in a gas of sodium atoms. *Phys. Rev. Lett.* **75**, 3969–3973 (1995).
- Andrews, M. R. *et al.* Observation of interference between two Bose condensates. *Science* **275**, 637–641 (1997).
- Burt, E. A. *et al.* Coherence, correlations, and collisions: What one learns about Bose-Einstein condensates from their decay. *Phys. Rev. Lett.* **79**, 337–340 (1997).
- Bloch, I. *et al.* Measurement of the spatial coherence of a trapped Bose gas at the phase transition. *Nature* **403**, 166–170 (2000).
- Moskalenko, S. A. Reversible optico-hydrodynamic phenomena in a non ideal exciton gas. *Sov. Phys. Solid State* **4**, 199–204 (1962).
- Blatt, J. M. Bose-Einstein condensation of excitons. *Phys. Rev.* **126**, 1691–1692 (1962).
- Keldysh, L. V. & Kozlov, A. N. Collective properties of excitons in semiconductors. *Sov. Phys. JETP* **27**, 521–525 (1968).
- Snoke, D. W. Spontaneous Bose coherence of excitons and polaritons. *Science* **298**, 1368–1372 (2002).
- Snoke, D. W. When should we say we have observed Bose condensation of excitons? *Phys. Status Solidi B* **238**, 389–396 (2003).
- Rüegg, Ch. *et al.* Bose-Einstein condensation of the triplet states in the magnetic insulator TiCuCl_3 . *Nature* **423**, 62–65 (2003).
- Eisenstein, J. P. & MacDonald, A. H. Bose-Einstein condensation of excitons in bilayer electron systems. *Nature* **432**, 691–694 (2004).
- Butov, L. V. *et al.* Towards Bose-Einstein condensation of excitons in potential traps. *Nature* **417**, 47–52 (2002).
- Snoke, D. W. *et al.* Long-range transport in excitonic dark states in coupled quantum wells. *Nature* **418**, 754–757 (2002).
- Weisbuch, C. *et al.* Observation of the coupled exciton-photon mode splitting in a semiconductor quantum microcavity. *Phys. Rev. Lett.* **69**, 3314–3317 (1992).
- Kavokin, A. & Malpuech, G. *Cavity Polaritons* (Elsevier, Amsterdam, 2003).
- Savona, V. *et al.* Theory of polariton photoluminescence in arbitrary semiconductor microcavity structures. *Phys. Rev. B* **53**, 13051–13062 (1996).
- Le Si, D. *et al.* Stimulation of polariton photoluminescence in semiconductor microcavity. *Phys. Rev. Lett.* **81**, 3920–3923 (1998).
- Deng, H. *et al.* Condensation of semiconductor microcavity exciton polaritons. *Science* **298**, 199–202 (2002).
- Leggett, A. J. Bose-Einstein condensation in the alkali gases: Some fundamental concepts. *Rev. Mod. Phys.* **73**, 307–356 (2001).
- Pitaevskii, L. & Stringari, S. *Bose-Einstein Condensation* Ch. 1, 6, 15 (Oxford Science Publication, Oxford Univ. Press, Oxford, 2003).
- Mermin, N. D. & Wagner, H. Absence of ferromagnetism or antiferromagnetism in one- or two-dimensional isotropic Heisenberg models. *Phys. Rev. Lett.* **17**, 1133–1136 (1966).
- Keeling, J. *et al.* Polariton condensation with localized excitons and propagating photons. *Phys. Rev. Lett.* **93**, 226403 (2004).
- Marchetti, F. M. *et al.* Thermodynamics and excitations of condensed polaritons in disordered microcavities. *Phys. Rev. Lett.* **96**, 066405 (2006).
- Szymańska, M. H. *et al.* Non-equilibrium quantum condensation in an incoherently pumped dissipative system. *Phys. Rev. Lett.* **96**, 230602 (2006).
- André, R. *et al.* Spectroscopy of polaritons in CdTe-based microcavities. *J. Cryst. Growth* **184/185**, 758–762 (1998).
- Bœuf, F. *et al.* Evidence of polariton stimulation in semiconductor microcavities. *Phys. Rev. B* **62**, R2279–R2282 (2000).
- Richard, M. *et al.* Spontaneous coherent phase transition of polaritons in CdTe microcavities. *Phys. Rev. Lett.* **94**, 187401 (2005).
- Richard, M. *et al.* Experimental evidence for nonequilibrium Bose condensation of exciton polaritons. *Phys. Rev. B* **72**, 201301(R) (2005).
- Bloch, J. *et al.* Monitoring the dynamics of a coherent cavity polariton population. *Phys. Rev. B* **71**, 155311 (2005).
- Porrás, D. & Tejedor, C. Linewidth of a polariton laser: Theoretical analysis of self-interaction effects. *Phys. Rev. B* **67**, 161310(R) (2003).
- Tassone, F. *et al.* Bottleneck effects in the relaxation and photoluminescence of microcavity polaritons. *Phys. Rev. B* **56**, 7554–7563 (1997).
- Müller, M. *et al.* Dynamics of the cavity polariton in CdTe-based semiconductor microcavities: Evidence for a relaxation edge. *Phys. Rev. B* **62**, 16886–16892 (2000).
- Ensher, J. R. *et al.* Bose-Einstein condensation in a dilute gas: Measurement of energy and ground state occupation. *Phys. Rev. Lett.* **77**, 4984–4987 (1996).
- Sokol, P. in *Bose-Einstein Condensation* (eds Griffin, A., Snoke, D. W. & Stringari, S.) 51 (Cambridge Univ. Press, Cambridge, 1995).
- Porrás, D. *et al.* Polariton dynamics and Bose-Einstein condensation in semiconductor microcavities. *Phys. Rev. B* **66**, 85304 (2002).
- Martin, M. D. *et al.* Striking dynamics of II-VI microcavity polaritons after linearly polarized excitation. *Phys. Status Solidi C* **2**, 3880–3883 (2005).
- Savvidis, P. G. *et al.* Angle-resonant stimulated polariton amplifier. *Phys. Rev. Lett.* **84**, 1547–1550 (2000).
- Ciuti, C. *et al.* Parametric luminescence of microcavity polaritons. *Phys. Rev. B* **63**, 041303(R) (2001).
- Laussy, F. P. *et al.* Effects of Bose-Einstein condensation of exciton polaritons in microcavities on the polarization of emitted light. *Phys. Rev. B* **73**, 035315 (2006).
- Imamoglu, A. & Ram, R. J. Quantum dynamics of exciton lasers. *Phys. Lett. A* **214**, 193–198 (1996).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements This work is dedicated to R. Romestain. We acknowledge support by the European Union Network "Photon-mediated phenomena in semiconductor nanostructures" and from the Swiss National Research Foundation through the "Quantum Photonics NCCR". We thank J.-P. Poizat, D. Sarchi and O. El Daïf for many discussions.

Author Contributions J.K., M.R., S.K. and A.B. contributed equally to this work: J.K. worked on 'Thermalization and condensation' and 'Linear polarization build-up'; M.R., S.K. and A.B. worked on the 'Long-range spatial coherence'. J.M.J.K., F.M.M. and M.H.S. performed theoretical modelling of the data and prepared the Supplementary Information.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to B.D. (benoit.deveaud-pledan@epfl.ch) and J.K. (jkasprz@spectro.ujf-grenoble.fr).

Evolution of alternative transcriptional circuits with identical logic

Annie E. Tsong^{1,2,*†}, Brian B. Tuch^{1,2,*}, Hao Li¹ & Alexander D. Johnson^{1,2}

Evolution of gene regulation is an important contributor to the variety of life. Here, we analyse the evolution of a combinatorial transcriptional circuit composed of sequence-specific DNA-binding proteins that are conserved among all eukaryotes. This circuit regulates mating in the ascomycete yeast lineage. We first identify a group of mating genes that was transcriptionally regulated by an activator in a fungal ancestor, but is now transcriptionally regulated by a repressor in modern bakers' yeast. Despite this change in regulatory mechanism, the logical output of the overall circuit remains the same. By examining the regulation of mating in modern yeasts that are related to different extents, we deduce specific, sequential changes in both *cis*- and *trans*-regulatory elements that constitute the transition from positive to negative regulation. These changes indicate specific mechanisms by which fitness barriers were traversed during the transition.

Darwinian evolution posits that natural selection, acting on heritable, random, 'successive, slight variations' in organisms over billions of years, can result in new biological features¹. Although recent work has revealed that biological novelty is often attributable to changes in transcriptional regulation^{2–6}, detailed analyses of such changes are often limited to a subset of the *cis*- or *trans*-elements involved^{7–13}. Here, we present a step-by-step analysis of evolution in a combinatorial transcriptional circuit that regulates mating in many yeast species of the ascomycete lineage.

Mating type in the yeasts *Saccharomyces cerevisiae* and *Candida albicans* is controlled by a segment of DNA called the MAT locus^{14–16}. The MAT locus exists in two versions, MAT α and MAT α , each of which encodes unique sequence-specific DNA-binding proteins that direct an extensive program of gene transcription. Cells that express only the MAT α - or MAT α -encoded DNA-binding proteins are α -cells and α -cells, respectively, and are specialized for mating. The α -cells express α -specific genes (*asgs*), which are required for α -cells to mate with α -cells. Likewise, α -cells express the α -specific genes (*asgs*). The third cell type, α/α , is formed when an α -cell mates with an α -cell. These cells do not mate, because the *asgs* and *asgs* are turned off (Fig. 1a).

Although this strategy is the same for both *S. cerevisiae* and *C. albicans*, the molecular details differ in a remarkable way¹⁶. In *S. cerevisiae*, the *asgs* are on by default, and are repressed in α - and α/α -cells by a homeodomain protein ($\alpha 2$) that is encoded by MAT α . In *C. albicans*, however, the *asgs* are off by default, and are activated in α -cells by an HMG-domain protein ($\alpha 2$) that is encoded by MAT α (Fig. 1a). Both molecular mechanisms give the same logical output: *asgs* are expressed only in α -cells. As the $\alpha 2$ -activation mode is found over a broad phylogenetic range of yeasts, this strategy most likely represents the ancestral state^{16–21} (Fig. 1b). By contrast, the $\alpha 2$ gene was recently lost in the *S. cerevisiae* lineage, which now uses the $\alpha 2$ -repressing mode of *asg* regulation²², indicating that $\alpha 2$ -mediated repression of *asgs* is a recent innovation.

The evolutionary transition from positive to negative regulation of the *asgs* has necessarily included at least two steps: (1) *asg* expression becoming independent of the activator $\alpha 2$, and (2) *asgs* coming under negative control by $\alpha 2$. We have used experimental and

informatic approaches to identify multiple changes in *cis*- and *trans*-elements that underlie these steps; we also infer the order in which these steps probably occurred.

Identification of ancestral α -specific genes

To understand how $\alpha 2$ came to repress the *asgs* in *S. cerevisiae*, we first sought the ancestral *cis*-element that was responsible for positive regulation of *asgs* by $\alpha 2$. We reasoned that extant yeasts that retain the ancestral regulatory logic, such as *C. albicans*, might also have retained *cis*-elements close to the ancestral form. *C. albicans*, a fungal pathogen of humans, last shared a common ancestor with *S. cerevisiae* 200–800 million years ago^{16,23,24}.

We first experimentally identified the *asgs* in *C. albicans* by comparing the transcriptional profiles of pheromone-induced α -cells to that of pheromone-induced α -cells (Fig. 2; for experimental details, see Methods and Supplementary Fig. 1)^{16,25,26}. This comparison revealed a group of six genes that were induced only in α -strains (Fig. 2c). Below, we show that expression of the gene *STE2* is also specific to α -cells. Of these seven genes, four have orthologues previously classified as α -specific in *S. cerevisiae* (*ASG7*, *BAR1*, *STE2* and *STE6*), indicating that they were α -specific in the common ancestor of *S. cerevisiae* and *C. albicans*.

Identification of *C. albicans* *asg* regulatory sequences

To identify *cis*-elements that are involved in activation by $\alpha 2$, we submitted *C. albicans* *asg* promoters (1,000 base pairs (bp)) to the motif-finding program MEME²⁷. In the promoters of six *asgs*, we found a regulatory element with several distinctive features (Fig. 3a). First, at 26-bp long, the element is more specified than the typical eukaryotic *cis*-acting sequence. Second, the sequence contains a region that closely resembles the binding site of Mcm1, a MADS box sequence-specific DNA-binding protein that is expressed equally in all three mating types, and is required for the regulation of both *asgs* and *asgs* in *S. cerevisiae*. The Mcm1 residues that contact DNA^{28,29} are fully conserved between *C. albicans* and *S. cerevisiae*, strongly implicating this region of the element as a binding site for Mcm1 in *C. albicans*. Third, the putative Mcm1 site in *C. albicans* *asg*

¹Department of Biochemistry & Biophysics, ²Department of Microbiology & Immunology, University of California San Francisco, San Francisco, California 94143-2200, USA.

[†]Present address: Department of Molecular and Cell Biology, University of California Berkeley, Lawrence Berkeley National Labs, 1 Cyclotron Road, Mailstop 84-355, Berkeley, California 94703, USA.

*These authors contributed equally to this work.

promoters lies next to a motif of the consensus sequence CATTGTC (Fig. 3a). The spacing between this motif and the Mcm1 site is always 4 bp. This motif is similar to demonstrated binding sites for $\alpha 2$ orthologues in *Schizosaccharomyces pombe* and *Neurospora crassa*, and to the $\alpha 2$ monomer site of *S. cerevisiae*^{20,30–32} (Fig. 3b).

Experimental validation of *C. albicans* asg regulatory sequence

To test whether the motif upstream of *C. albicans* asgs is functional, we fused a wild-type or mutant fragment of the *STE2* promoter to a green fluorescent protein (GFP) reporter³³ (Fig. 3c). In the mutant promoter, the conserved motif was mutated from CATTGTC to CATAATC, a change that is predicted to destroy the $\alpha 2$ -binding site. The wild-type promoter activated GFP on exposure to α -factor (Fig. 3c), whereas the mutant promoter showed no induction of GFP (Fig. 3d), demonstrating that this *cis*-element is required for $\alpha 2$ -dependent activation of asgs.

Analysis of *cis*-asg regulation across species

For ancestral asgs to undergo the transition from positive to negative regulation, $\alpha 2$ -bound *cis*-elements were probably lost, whereas

$\alpha 2$ -bound elements must have been gained. To investigate when this transition occurred, we first inferred a phylogeny of 16 yeast species whose genomes have been sequenced, then identified orthologues of the asgs of *C. albicans* and *S. cerevisiae* in all 16 yeasts^{34,35} (Fig. 4b, see Methods). Position-specific scoring matrices (PSSMs) constructed from the *S. cerevisiae* or *C. albicans* asg operators (Fig. 4a) were used to scan the promoters of each asg orthologue. Maximum log₁₀-odds scores are shown in Fig. 4c, d.

S. cerevisiae-like asg operators (an Mcm1 site flanked by two $\alpha 2$ -binding sites) were clearly found in orthologous promoters of organisms as far diverged as *Saccharomyces castellii*. In further diverged organisms, the presence of an *S. cerevisiae*-like asg operator was diminished, although it was found in some *Candida glabrata*, *Kluyveromyces lactis*, *Eremothecium gossypii* and *Kluyveromyces waltii* promoters (Fig. 4c). The *C. albicans* PSSM yielded a nearly converse pattern (Fig. 4d). Organisms that branch with *C. albicans* have *C. albicans*-like asg operators (an Mcm1 site flanked by a single $\alpha 2$ site); however, this matrix recovered no significant matches in species close to *S. cerevisiae*, correlating with the loss of $\alpha 2$ (ref. 22). These results are unchanged by recently proposed alternative phylogenetic topologies³⁶.

Identification of the asg operator in the *K. lactis* branch

Neither the *C. albicans* matrix nor that of *S. cerevisiae* elicited strong matches in the *K. lactis*-branch yeasts, which share a more recent common ancestor with *S. cerevisiae* than does *C. albicans* (Fig. 4b). To determine independently whether this lineage has a unique asg operator, we submitted promoters of the ancestral asg orthologues (*ASG7*, *BAR1*, *STE2* and *STE6*) from the *K. lactis* branch yeasts to MEME. The highest scoring hit was a DNA motif with features in

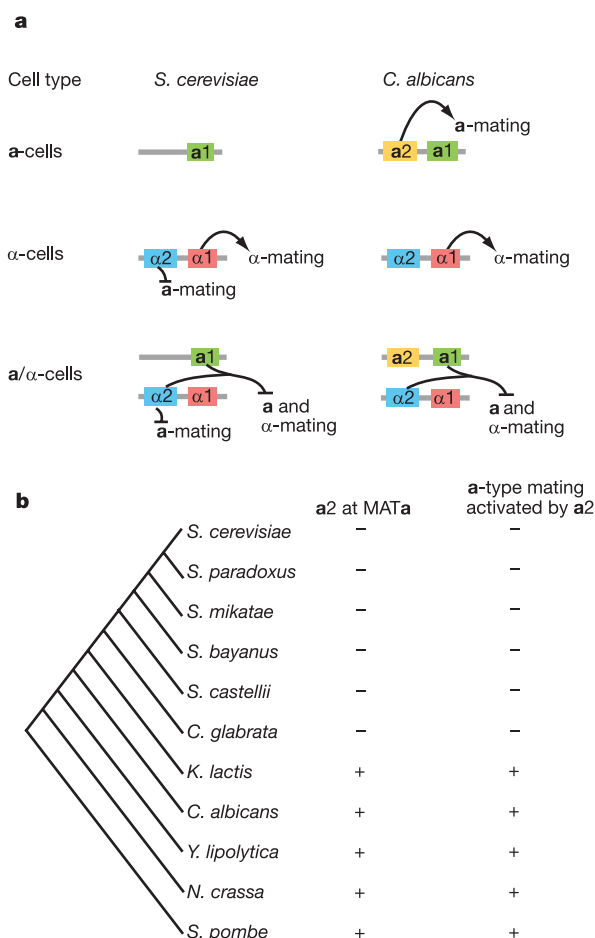


Figure 1 | a-type mating is negatively regulated in modern *S. cerevisiae*, but was positively regulated in its ancestor. **a**, *S. cerevisiae* and *C. albicans* transcribe their genes according to one of three programs, which produce the α -, α - and a/ α -cells. The particular cell type produced is determined by the MAT locus, which encodes sequence-specific DNA-binding proteins (coloured blocks). Regulation of a-type mating differs substantially between *S. cerevisiae* and *C. albicans*. In *S. cerevisiae*, a-type mating is repressed in α -cells by $\alpha 2$. In *C. albicans*, a-type mating is activated in α -cells by $\alpha 2$. In both organisms, α -cells mate with α -cells to form a/ α -cells, which cannot mate. **b**, $\alpha 2$ is an activator of a-type mating over a broad phylogenetic range of yeasts^{16,18–21,47}. In *S. cerevisiae* and close relatives, $\alpha 2$ has taken over regulation of the asgs²².

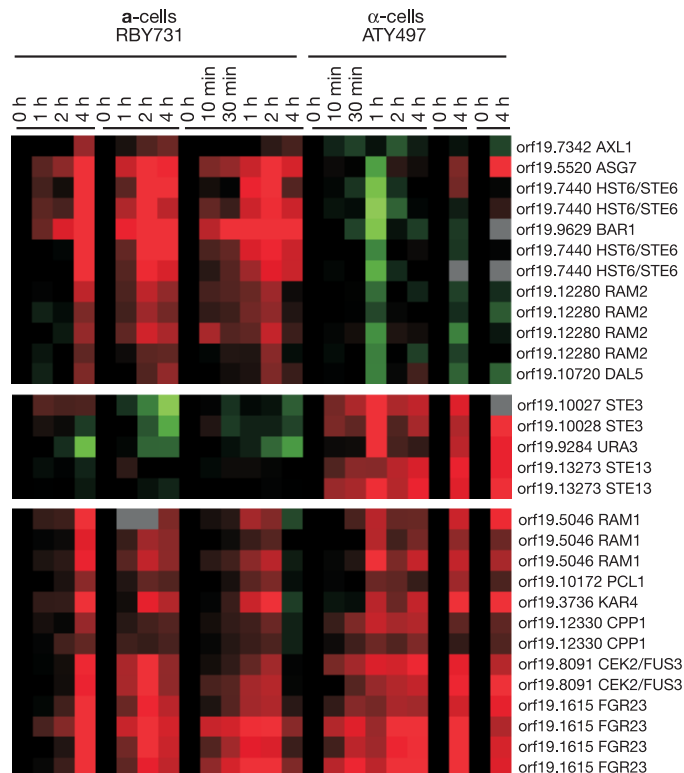


Figure 2 | Identification of a-specific genes in *C. albicans*. Pheromone induction profiles of a-cells (RBY731) and α -cells (ATY497) in six pheromone induction time-courses are compared. Top: genes upregulated only in a-cells. Middle: genes upregulated only in α -cells. *URA3* is induced because it is under the control of the *STE3* promoter. Bottom: subset of genes upregulated in both a- and α -cells. The first two time-courses were previously published by Bennett *et al.*²⁶.

common with both the *S. cerevisiae* and *C. albicans* *asg* operators, indicating that it might be a transitional form. As in *C. albicans*, this motif contains an Mcm1 site flanked by an *a2* site on one side. However, it is also defined on the opposite side, resembling the tripartite structure of the *S. cerevisiae* operator. This additional sequence information is similar to both the *S. cerevisiae* $\alpha 2$ - and *C. albicans* *a2*-site consensus binding sequences; moreover, the spacing from the Mcm1 binding sequence is also similar to that found in *S. cerevisiae* and *C. albicans* *asg* operators (Fig. 4e). An independent clustering analysis of putative *asg* operators supports the idea that there is a transitional form in the *K. lactis* branch (see Supplementary Fig. 2).

Because of low genome sequence coverage³⁶ we did not systematically incorporate the yeast *Saccharomyces kluyveri*, which branches near *K. lactis* and retains *a2* (refs 11, 22, 37, 38), into our studies. However, the available sequences of *asg* promoters from *S. kluyveri* also contain operators similar to those of the *K. lactis* branch (not shown), indicating that transitional forms of the operator might also exist in this species.

Emergence of the $\alpha 2$ -Mcm1 interaction

Repression of the *asgs* in *S. cerevisiae* requires a cooperative interaction between the *trans*-factors $\alpha 2$ and Mcm1. To determine when this interaction arose, we aligned orthologues of $\alpha 2$ and Mcm1 across several yeast species, then searched for conservation of the interaction interface^{28,29,39} (Fig. 5a, b). The region of Mcm1 that contacts $\alpha 2$ is highly conserved across all species analysed (Fig. 5a). Many proteins besides $\alpha 2$ contact this region, so the high degree of conservation is not surprising. By contrast, the portion of $\alpha 2$ that contacts Mcm1 varies considerably across yeasts (Fig. 5b). A critical nine-residue 'linker' region that is required for the interaction between $\alpha 2$ and Mcm1 in *S. cerevisiae*³⁹ is highly conserved from *S. cerevisiae* to *C. glabrata*, and is also somewhat conserved in *K. lactis* and *S. kluyveri*; however, this region shows no conservation in yeasts that branch with *C. albicans*,

consistent with observations that $\alpha 2$ is not involved in *asg* expression in *C. albicans*¹⁶ (Fig. 1a).

Structural homology modelling of *K. lactis* $\alpha 2$ and Mcm1 using the *S. cerevisiae* crystal structure³⁹ as a template shows that, despite several substitutions, the $\alpha 2$ -Mcm1 interaction interfaces in *K. lactis* are fully compatible⁴⁰ (Fig. 5c); thus, the appearance of the $\alpha 2$ -Mcm1 interaction coincides with the emergence of the tripartite, *S. cerevisiae*-like *asg* operator in the *K. lactis* branch (Fig. 4). This suggests that the *K. lactis* *asg* operators are bound by $\alpha 2$ -Mcm1. We also know that *K. lactis* *a2* is required for wild-type levels of *a*-type mating (A.E.T., unpublished work). Together, our data indicate that *K. lactis* *asgs* are controlled by both $\alpha 2$ and *a2* through one of three possible scenarios: (1) some operators are bound exclusively by *a2*-Mcm1 and others are bound exclusively by $\alpha 2$ -Mcm1, (2) hybrid operators are bound by both *a2*-Mcm1 and $\alpha 2$ -Mcm1, or (3) a combination of these.

Discussion

In this work, we identify a group of genes (the *asgs*) that was positively regulated in an ancestral yeast, but is negatively regulated in modern *S. cerevisiae*. Orthologues of these genes are required for sexual differentiation in fungal lineages that are proposed to span up to 1.3 billion years of evolution^{24,41}. We identify specific changes in *cis*- and *trans*-elements that underlie the two critical steps in this transition: (1) *asg* expression becoming independent of the activator *a2*, and (2) *asg* expression coming under negative control of $\alpha 2$. The nature of these changes provides a plausible explanation for how fitness barriers were overcome during the regulatory transition, both in terms of the smaller-scale challenges of evolving individual protein-protein and protein-DNA interactions, and as regards the larger-scale challenge of maintaining appropriate *asg* regulation throughout the transition.

Independence of *asg* expression from the activator *a2*. During the transition from positive to negative regulation of the *asgs*, *asg* expression became independent of the activator *a2*. We have

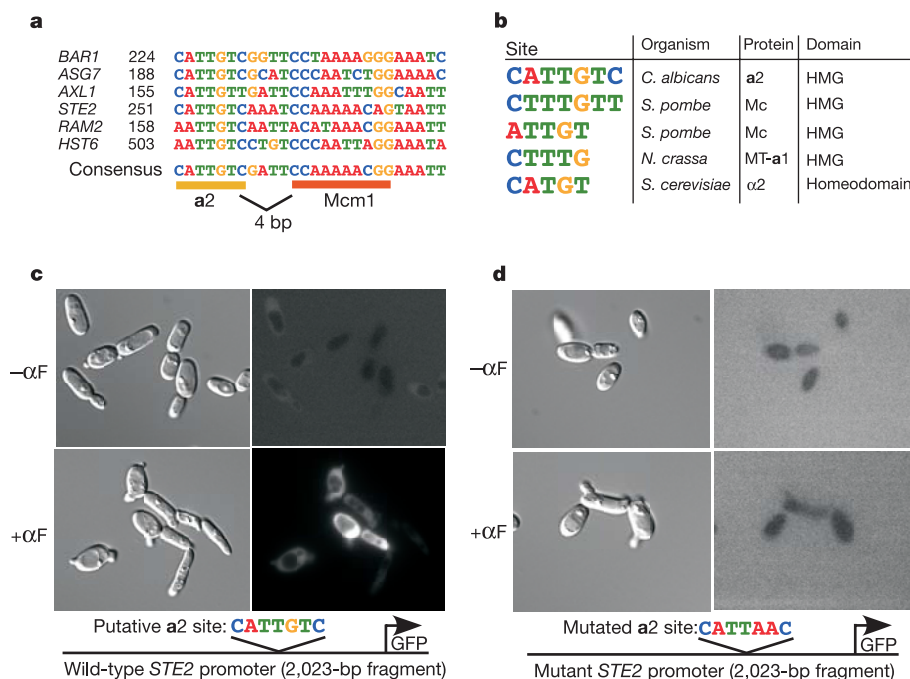


Figure 3 | Identification and validation of the *C. albicans* *asg* operator.

a, 1,000 bp of each *C. albicans* *asg* promoter were submitted to MEME²⁷. The motif shown was present in six *asg* promoters. Distance from the translation start site is indicated. The element contains a conserved 7-bp site (yellow) and a putative Mcm1 binding site (orange), separated by 4 bp. **b**, The 7-bp motif is similar to binding sites of *a2* orthologues from *N. crassa* and

S. pombe, as well as $\alpha 2$ from *S. cerevisiae*^{20,30–32}. *S. pombe* MatMc binds the two indicated sites equally³⁰. **c**, **d**, A wild-type (**c**) or mutant (**d**) 2,023-bp fragment of the *STE2* promoter was fused to a GFP reporter and integrated at the RP10 locus of *C. albicans*³³. Top panels: uninduced cells. Bottom panels: α -factor induction. Only the wild-type *STE2* promoter activates GFP expression (bottom right panels).

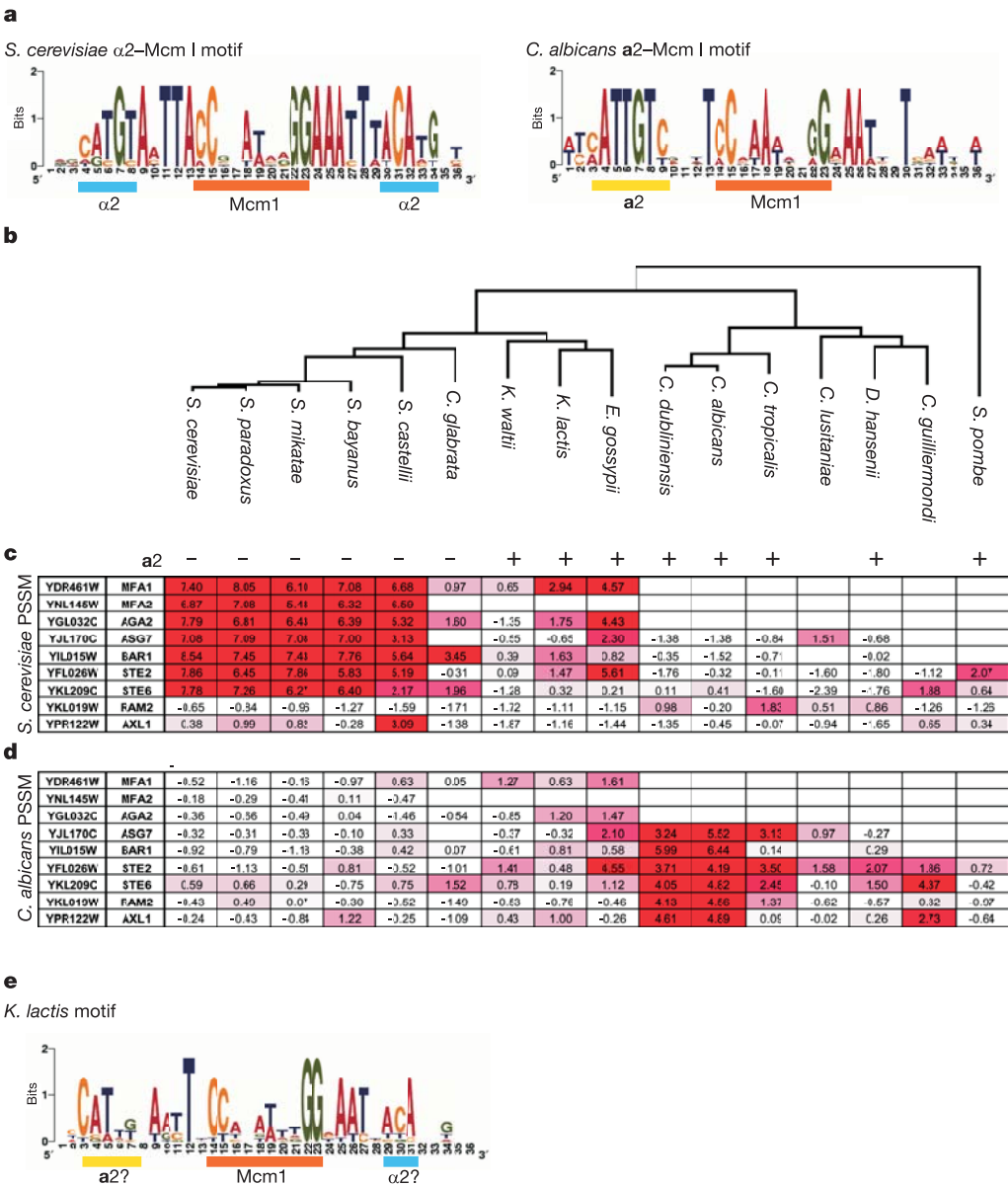


Figure 4 | Analysis of *cis*- *asg* regulation across species. **a**, *S. cerevisiae* $\alpha 2$ -Mcm1 and *C. albicans* $a 2$ -Mcm1 position-specific scoring matrices (PSSMs) were derived from the seven *S. cerevisiae* *asg* operators, or six *C. albicans* *asg* operators. **b**, A phylogeny of 16 sequenced yeasts was inferred using methods similar to those of Rokas *et al.*³⁴ *asg* orthologue promoters were scanned with the *S. cerevisiae* PSSM (**c**) or *C. albicans* PSSM (**d**).

shown that the transcriptional regulator Mcm1 was present at ancestral *asg* promoters as a co-activator with $a 2$; in *S. cerevisiae*, Mcm1 is also present at *asg* promoters, serving as both an activator (on its own) and a co-repressor (with $\alpha 2$). In *S. cerevisiae*, high A/T content surrounding the Mcm1 binding site allows Mcm1 to function without a cofactor⁴². Therefore, a simple increase in the A/T content flanking Mcm1 sites in *S. cerevisiae* *asg* operators is far higher than that flanking Mcm1 sites in *C. albicans* *asg* operators (Fig. 4a). **Establishment of *asg* repression by $\alpha 2$.** On its own, an increase in A/T content flanking the Mcm1 site would lead to inappropriate constitutive activation of the *asgs*, as Mcm1 is expressed equally in all cell types. However, *asg* regulation could be maintained if this increase were accompanied by evolution of $\alpha 2$ -mediated repression.

Maximum log₁₀-odds scores are shown. Darker shades of red indicate stronger matches. **e**, Promoters from the *K. waltii*, *K. lactis* and *E. gossypii* orthologues of *ASG7*, *BAR1*, *STE2* and *STE6* were pooled and submitted to MEME²⁷. The recovered motif has elements of both the *S. cerevisiae* and *C. albicans* *asg* operators: an $a 2$ -like site resembles that of *C. albicans*, and the tripartite structure resembles the *S. cerevisiae* operator.

Indeed, this is precisely what we observe: *cis*- and *trans*-changes, signifying the emergence of $\alpha 2$ -mediated repression in the *K. lactis* branch, accompany the increase in A/T content surrounding the Mcm1 site (Figs 4e, 5). Previous involvement of Mcm1 in *asg* regulation probably assisted in the evolution of $\alpha 2$ -mediated repression by increasing the number of surfaces available for $\alpha 2$ -promoter interaction to include both protein and DNA. The similarity of the $a 2$ -binding site (CATTGTC) to the $\alpha 2$ -binding site (CATGT), in both sequence and spacing from the Mcm1 site, no doubt contributed to the evolution of the *S. cerevisiae* *asg* operator (Figs 3, 4); a small change to the *cis*-element could convert it from an $a 2$ - to an $\alpha 2$ -recognition sequence. The similarity of the sites is particularly striking, given that $a 2$ and $\alpha 2$ belong to different protein families (the HMG and homeodomain families, respectively). **Ordering the pathway.** An important clue as to the order in which individual *cis*- and *trans*- changes occurred comes from *K. lactis* and

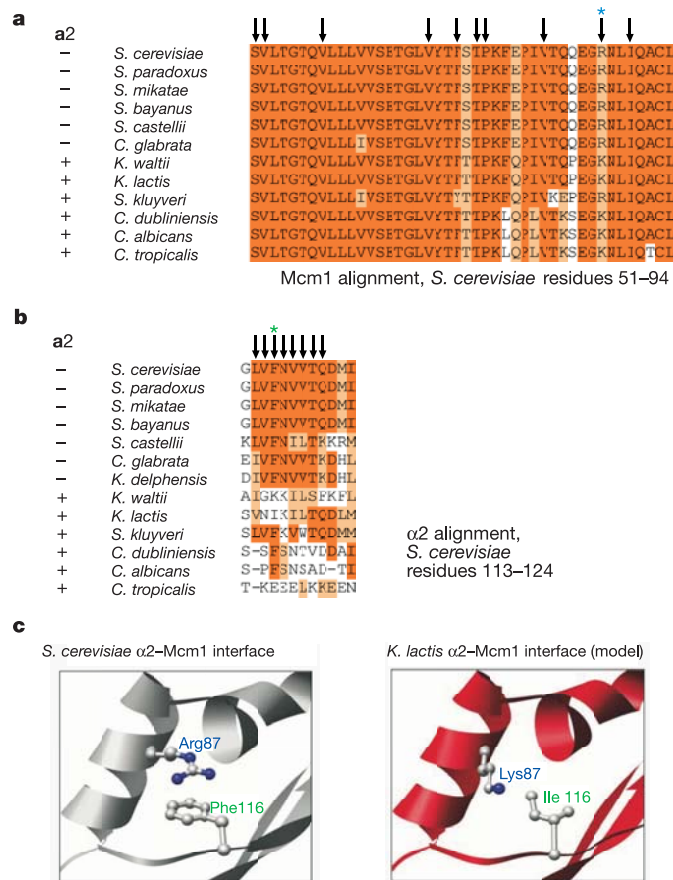


Figure 5 | Evolution of the α2-Mcm1 interaction. **a**, Mcm1 sequences from 12 species are aligned. Arrows denote residues of Mcm1 that contact α2 in *S. cerevisiae*^{28,29}. **b**, α2 sequences from 13 species are aligned. Arrows indicate residues of α2 that contact Mcm1, and are required for α2-Mcm1 repression^{29,39}. This region is well conserved out to *C. glabrata*, with *K. lactis* and *S. kluyveri* α2 also showing significant conservation. **c**, The *K. lactis* α2-Mcm1 complex was modelled using the crystal structure of the *S. cerevisiae* α2-Mcm1 complex (PDB ID: 1MNM; Tan S 1998) as a template. Left: *S. cerevisiae* α2 linker region and Mcm1 interface. Mcm1-Arg87 (blue asterisk of **a**) and α2-Phe116 (green asterisk of **b**) form a favourable pi-stacking interaction. Right: *K. lactis* model. The Arg87-Phe116 interaction is not present, indicating that the *K. lactis* interaction is probably weaker than that of *S. cerevisiae*.

S. kluyveri. Both yeasts have retained a2 at their MATa loci, but in both yeasts an α2-Mcm1 interaction interface and a tripartite asg operator similar to the *S. cerevisiae* α2-Mcm1 binding site have emerged. By examining the data in a phylogenetic context (Fig. 6d), we can tentatively define the succession of events leading to repression of asgs in modern *S. cerevisiae* as follows. First, a2-Mcm1 activated asgs in an ancestor (Fig. 6a, d). Subsequently, the α2-Mcm1 protein interaction evolved, coincident with evolution of an α2 site and a strengthening of the Mcm1 binding site in the asg operator (Fig. 6b, d). After the divergence of *K. lactis*, the α2-Mcm1 cis-operator specificity and A/T content were increased, and a2 was lost, completing the hand-off from positive to negative control (Fig. 6c, d). A crucial feature of this model is that asgs are appropriately regulated throughout each stage of circuit evolution, a condition made possible by the continued presence of Mcm1. Intriguingly, both the loss of a2 and the conversion of asg regulation to an exclusively negative regulatory scheme coincide with a whole-genome duplication^{43,44}. The evolution of the asg regulatory circuit might have been facilitated in part by greater flexibility in asg regulation conferred by duplication of its component cis- and trans-elements.

Conclusion. Our analysis shows how a concerted series of subtle

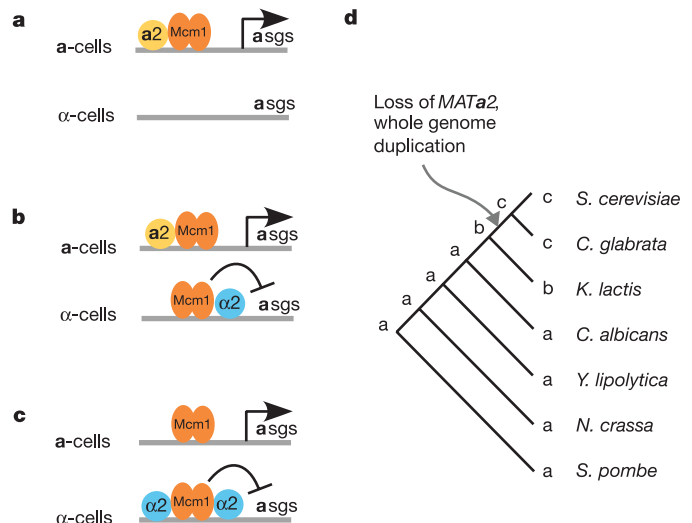


Figure 6 | Ordering the changes in cis- and trans-regulatory elements. **a**, In an ancestral yeast, a2-Mcm1 activated asgs in a-cells. This scheme persists in modern *C. albicans*. **b**, cis- and trans-elements in the *K. lactis* branch suggest that asgs are positively regulated by a2-Mcm1 in a-cells and negatively regulated by α2-Mcm1 in α-cells. **c**, In modern *S. cerevisiae*, asgs are activated by Mcm1 in a-cells and repressed by α2-Mcm1 in α-cells. **d**, The regulatory schemes shown in **a–c** are mapped onto extant species and ancestral nodes. Species from *C. albicans* to *S. pombe* most closely resemble **a**^{16–19}, whereas *K. lactis* fits **b** and *S. cerevisiae* and *C. glabrata* fit **c**. The most parsimonious evolutionary scenario maps scheme **a** as the ancestral state. Scheme **b** is transitional, first appearing in the ancestor of *K. lactis* and *S. cerevisiae*. Scheme **c** is the most derived, appearing in the ancestor of *C. glabrata* and *S. cerevisiae*.

changes in cis- and trans-elements can lead to a profound evolutionary change in the wiring of a combinatorial circuit. These changes include: (1) ‘tuning up’ of a binding site for a ubiquitous activator, making gene expression independent of a cell-type-specific activator; (2) a small change in an existing DNA-binding site, converting its recognition from one protein to that of an unrelated protein; (3) a small change in the amino-acid sequence of a sequence-specific DNA-binding protein, allowing it to bind DNA cooperatively with a second protein. Significantly, the coordinated optimization of protein–DNA and protein–protein interactions that we have described allows regulation of the target genes to be maintained throughout a major evolutionary transition. Because the proteins that have participated in this transition represent several highly conserved and prominent protein families, including the MADS box family (Mcm1), the HMG-domain family (a2) and the homeo-domain family (α2), the types of change we have described are likely to apply to other examples of transcriptional circuit evolution.

METHODS

Detailed information on Methods is described in the Supplementary Information.

Strain construction. The pheromone a-factor has not yet been identified in *C. albicans*. To compare the pheromone response of a-cells to that of α-cells, we ‘fooled’ α-cells into responding to α-factor by ectopically expressing the α-factor receptor (strain ATY497), a strategy previously employed in *S. cerevisiae*²⁵. Constructs and primers used are listed in Supplementary Information.

Induction of α-factor. Strains were grown to an optical density (OD₆₀₀) of 1.0 in YEPD plus 55 μg ml^{−1} adenine, then induced with 10 μg ml^{−1} α-factor from a stock dissolved in either dimethylsulphoxide (DMSO) or water. Sample preparation and microarrays were as previously described²⁶. All microarray data are available online (http://genome.ucsf.edu/asg_evolution/).

Yeast phylogeny. Briefly, groups of orthologous genes (see Supplementary Information) with one and only one representative from each of the 16 yeasts were multiply aligned with ClustalW⁴⁵, then concatenated to yield a single alignment. A maximum-likelihood species tree was inferred from this alignment using the TREE-PUZZLE algorithm⁴⁶. Trees with identical topologies were also

generated using additional algorithms (see Supplementary Information).

Structural modelling. The *K. lactis* $\alpha 2$ -Mcm1 interaction was modelled using the Protein Local Optimization Program, by M. P. Jacobson, Department of Pharmaceutical Chemistry, University of California San Francisco, USA (<http://francisco.compbio.ucsf.edu/~jacobson/>), using the crystal structure of the *S. cerevisiae* $\alpha 2$ -Mcm1 complex (PDB ID: 1NMN; Tan S 1998) as a template.

Received 24 February; accepted 21 July 2006.

- Darwin, C. R. *The Origin of Species* (Gramercy, New York, 1859).
- Carroll, S. B., Grenier, J. K. & Weatherbee, S. D. *From DNA to Diversity* (Blackwell Science, Malden, Massachusetts, 2001).
- Davidson, E. H. *Genomic Regulatory Systems* (Academic, San Diego, California, 2001).
- Gerhart, J. & Kirschner, M. *Cells, Embryos, and Evolution* (Blackwell Science, Malden, Massachusetts, 1997).
- Levine, M. & Tjian, R. Transcription regulation and animal diversity. *Nature* **424**, 147–151 (2003).
- Doebley, J. & Lukens, L. Transcriptional regulators and the evolution of plant form. *Plant Cell* **10**, 1075–1082 (1998).
- Gompel, N., Prud'homme, B., Wittkopp, P. J., Kassner, V. A. & Carroll, S. B. Chance caught on the wing: cis-regulatory evolution and the origin of pigment patterns in *Drosophila*. *Nature* **433**, 481–487 (2005).
- Ihmels, J. *et al.* Rewiring of the yeast transcriptional network through the evolution of motif usage. *Science* **309**, 938–940 (2005).
- Ludwig, M. Z., Patel, N. H. & Kreitman, M. Functional analysis of eve stripe 2 enhancer evolution in *Drosophila*: rules governing conservation and change. *Development* **125**, 949–958 (1998).
- Ronshaugen, M., McGinnis, N. & McGinnis, W. Hox protein mutation and macroevolution of the insect body plan. *Nature* **415**, 914–917 (2002).
- Tanay, A., Reggev, A. & Shamir, R. Conservation and evolvability in regulatory networks: the evolution of ribosomal regulation in yeast. *Proc. Natl Acad. Sci. USA* **102**, 7203–7208 (2005).
- Wittkopp, P. J., Haerum, B. K. & Clark, A. G. Evolutionary changes in cis and trans gene regulation. *Nature* **430**, 85–88 (2004).
- Galant, R. & Carroll, S. B. Evolution of a transcriptional repression domain in an insect Hox protein. *Nature* **415**, 910–913 (2002).
- Hull, C. M., Raisner, R. M. & Johnson, A. D. Evidence for mating of the "asexual" yeast *Candida albicans* in a mammalian host. *Science* **289**, 307–310 (2000).
- Herskowitz, I., Rine, J. & Strathern, J. *Mating-type determination and Mating-type interconversion in Saccharomyces cerevisiae* (eds Jones, E. W., Pringle, J. R. & Broach, J. R.) 583–656 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1992).
- Tsong, A. E., Miller, M. G., Raisner, R. M. & Johnson, A. D. Evolution of a combinatorial transcriptional circuit: a case study in yeasts. *Cell* **115**, 389–399 (2003).
- Staben, C. & Yanofsky, C. *Neurospora crassa* a mating-type region. *Proc. Natl Acad. Sci. USA* **87**, 4917–4921 (1990).
- Kelly, M., Burke, J., Smith, M., Klar, A. & Beach, D. Four mating-type genes control sexual differentiation in the fission yeast. *EMBO J.* **7**, 1537–1547 (1988).
- Kurischko, C., Schilhabel, M. B., Kunze, I. & Franzl, E. The MATA locus of the dimorphic yeast *Yarrowia lipolytica* consists of two divergently oriented genes. *Mol. Gen. Genet.* **262**, 180–188 (1999).
- Philly, M. L. & Staben, C. Functional analyses of the *Neurospora crassa* MT a-1 mating type polypeptide. *Genetics* **137**, 715–722 (1994).
- Turgeon, B. G. *et al.* Cloning and analysis of the mating type genes from *Cochliobolus heterostrophus*. *Mol. Gen. Genet.* **238**, 270–284 (1993).
- Butler, G. *et al.* Evolution of the MAT locus and its Ho endonuclease in yeast species. *Proc. Natl Acad. Sci. USA* **101**, 1632–1637 (2004).
- Calderone, R. A. *Candida and Candidiasis* (ed. Calderone, R. A.) (ASM, Washington DC, 2002).
- Hedges, S. B. The origin and evolution of model organisms. *Nature Rev. Genet.* **3**, 838–849 (2002).
- Bender, A. & Sprague, G. F. Jr. Yeast peptide pheromones, a-factor and α -factor, activate a common response mechanism in their target cells. *Cell* **47**, 929–937 (1986).
- Bennett, R. J., Uhl, M. A., Miller, M. G. & Johnson, A. D. Identification and characterization of a *Candida albicans* mating pheromone. *Mol. Cell. Biol.* **23**, 8189–8201 (2003).
- Bailey, T. L. & Elkan, C. Fitting a mixture model by expectation maximization to discover motifs in biopolymers. *Proc. Int. Conf. Intell. Syst. Mol. Biol.* **2**, 28–36 (1994).
- Acton, T. B., Mead, J., Steiner, A. M. & Vershon, A. K. Scanning mutagenesis of Mcm1: residues required for DNA binding, DNA bending, and transcriptional activation by a MADS-box protein. *Mol. Cell. Biol.* **20**, 1–11 (2000).
- Tan, S. & Richmond, T. J. Crystal structure of the yeast MAT α 2/MCM1/DNA ternary complex. *Nature* **391**, 660–666 (1998).
- Kjaerulff, S., Dooijes, D., Clevers, H. & Nielsen, O. Cell differentiation by interaction of two HMG-box proteins: Mat1-Mc activates M cell-specific genes in *S. pombe* by recruiting the ubiquitous transcription factor Ste11 to weak binding sites. *EMBO J.* **16**, 4021–4033 (1997).
- Smith, D. L. & Johnson, A. D. A molecular mechanism for combinatorial control in yeast: MCM1 protein sets the spacing and orientation of the homeodomains of an $\alpha 2$ dimer. *Cell* **68**, 133–142 (1992).
- van Beest, M. *et al.* Sequence-specific high mobility group box factors recognize 10–12-base pair minor groove motifs. *J. Biol. Chem.* **275**, 27266–27273 (2000).
- Care, R. S., Trevethick, J., Binley, K. M. & Sudbery, P. E. The MET3 promoter: a new tool for *Candida albicans* molecular genetics. *Mol. Microbiol.* **34**, 792–798 (1999).
- Rokas, A., Williams, B. L., King, N. & Carroll, S. B. Genome-scale approaches to resolving incongruence in molecular phylogenies. *Nature* **425**, 798–804 (2003).
- Byrne, K. P. & Wolfe, K. H. The Yeast Gene Order Browser: combining curated homology and syntenic context reveals gene fate in polyploid species. *Genome Res.* **15**, 1456–1461 (2005).
- Cliften, P. *et al.* Finding functional features in *Saccharomyces* genomes by phylogenetic footprinting. *Science* **301**, 71–76 (2003).
- Belloch, C., Querol, A., Garcia, M. D. & Barrio, E. Phylogeny of the genus *Kluyveromyces* inferred from the mitochondrial cytochrome-c oxidase II gene. *Int. J. Syst. Evol. Microbiol.* **50**, 405–416 (2000).
- Wong, S., Butler, G. & Wolfe, K. H. Gene order evolution and paleopolyploidy in hemiascomycete yeasts. *Proc. Natl Acad. Sci. USA* **99**, 9272–9277 (2002).
- Mead, J., Zhong, H., Acton, T. B. & Vershon, A. K. The yeast $\alpha 2$ and Mcm1 proteins interact through a region similar to a motif found in homeodomain proteins of higher eukaryotes. *Mol. Cell. Biol.* **16**, 2135–2143 (1996).
- Jacobson, M. P. & Friesner, R. A. Protein local optimization program. (http://francisco.compbio.ucsf.edu/~jacobson/plop_manual/plop_overview.htm) (2006).
- Lengeler, K. B. *et al.* Signal transduction cascades regulating fungal development and virulence. *Microbiol. Mol. Biol. Rev.* **64**, 746–785 (2000).
- Acton, T. B., Zhong, H. & Vershon, A. K. DNA-binding specificity of Mcm1: operator mutations that alter DNA-bending and transcriptional activities by a MADS box protein. *Mol. Cell. Biol.* **17**, 1881–1889 (1997).
- Kellis, M., Birren, B. W. & Lander, E. S. Proof and evolutionary analysis of ancient genome duplication in the yeast *Saccharomyces cerevisiae*. *Nature* **428**, 617–624 (2004).
- Wolfe, K. H. & Shields, D. C. Molecular evidence for an ancient duplication of the entire yeast genome. *Nature* **387**, 708–713 (1997).
- Higgins, D. G. & Sharp, P. M. CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. *Gene* **73**, 237–244 (1988).
- Schmidt, H. A., Strimmer, K., Vingron, M. & von Haeseler, A. TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. *Bioinformatics* **18**, 502–504 (2002).
- Debuchy, R., Arnaise, S. & Lecellier, G. The *mat*⁺ allele of *Podospora anserina* contains three regulatory genes required for the development of fertilized female organs. *Mol. Gen. Genet.* **241**, 667–673 (1993).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We are grateful to M. Jacobson for advice provided in modelling the *K. lactis* $\alpha 2$ and Mcm1 structures. We also thank S. Åström for providing unpublished *K. lactis* strains and advice on their handling, P. Sudbury for providing the GFP reporter construct, M. Lorentz and G. Fink for the collaboration that produced the DNA microarrays used in this paper, and the Broad Institute (<http://www.broad.mit.edu/annotation/fungi/fgi/>), the Sanger Center (<http://www.sanger.ac.uk/Projects/Fungi/>), and the Pathogen Sequencing Unit at the Wellcome Trust Sanger Institute (<http://www.sanger.ac.uk/sequencing/Candida/dubliniensis/>) for making sequence data available. M. Ptashne provided valuable comments on the manuscript. We thank B. Hromatka for overseeing microarray printing, and R. Bennett for microarray data and discussions. R. Zordan, A. Uhl, M. Lohse, M. Miller, R. Wu, C. Chaiworapal and other members of the Johnson and Li labs provided useful discussions. This work was supported by grants from the NIH to A.D.J. A.E.T. was supported by a Howard Hughes Medical Institute Predoctoral Fellowship. B.B.T. is an NSF Predoctoral Fellow. B.B.T. and H.L. acknowledge partial support from a Packard Fellowship in Science and Engineering (to H.L.) and an NIH grant.

Author Contributions A.E.T. determined the asgs of *C. albicans*, and validated the asg operator site. B.T. constructed the phylogenetic tree, analysed asg operators across multiple species, and modelled the *K. lactis* $\alpha 2$ -Mcm1 interaction. H.L. and A.D.J. oversaw the work.

Author Information Microarray data are available in Supplementary Information and at http://genome.ucsf.edu/asg_evolution/. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to A.D.J. (ajohnson@cgl.ucsf.edu).

Stem-cell ageing modified by the cyclin-dependent kinase inhibitor $p16^{INK4a}$

Viktor Janzen^{1,2*}, Randolph Forkert^{1,2*}, Heather E. Fleming^{1,2}, Yoriko Saito^{1,2}, Michael T. Waring¹, David M. Dombkowski¹, Tao Cheng^{1†}, Ronald A. DePinho³, Norman E. Sharpless⁴ & David T. Scadden^{1,2}

Stem-cell ageing is thought to contribute to altered tissue maintenance and repair. Older humans experience increased bone marrow failure and poorer haematologic tolerance of cytotoxic injury. Haematopoietic stem cells (HSCs) in older mice have decreased per-cell repopulating activity, self-renewal and homing abilities, myeloid skewing of differentiation, and increased apoptosis with stress. Here we report that the cyclin-dependent kinase inhibitor $p16^{INK4a}$, the level of which was previously noted to increase in other cell types with age, accumulates and modulates specific age-associated HSC functions. Notably, in the absence of $p16^{INK4a}$, HSC repopulating defects and apoptosis were mitigated, improving the stress tolerance of cells and the survival of animals in successive transplants, a stem-cell-autonomous tissue regeneration model. Inhibition of $p16^{INK4a}$ may ameliorate the physiological impact of ageing on stem cells and thereby improve injury repair in aged tissue.

Accompanying older age is a decreased ability to respond to stress on an organismal and tissue level. Linking these features to stem-cell-based functions has generally been indirect and of unknown molecular basis. Cyclin-dependent kinase inhibitors have been shown to play an important part in stem-cell regulation^{1–3}. The mammalian *INK4a/ARF* locus (*Cdkn2a*) encodes two linked tumour suppressor proteins: the cyclin-dependent kinase inhibitor $p16^{INK4a}$ and ARF, a potent regulator of p53 stability. The two open reading frames encoding $p16^{INK4a}$ and ARF have different promoters and first exons that splice into alternative reading frames in the shared second exon, generating two functionally unrelated, cancer-relevant proteins^{4,5}. $p16^{INK4a}$ expression is virtually undetectable in the tissues of young humans and rodents, although it increases with age and is associated with replicative senescence in cell lines^{6–8}. Whether senescence contributes to ageing *in vivo* is controversial. There are changes in HSCs with age that include an overall increase in HSC number in some mouse strains (for example, C57BL/6) and a decline in multiple functional parameters^{9–12}. We proposed that age-dependent changes in stem-cell function may, in part, be due to stem-cell-autonomous effects of $p16^{INK4a}$.

$p16^{INK4a}$ expression in HSCs increases with age

Expression of $p16^{INK4a}$ was examined in different subpopulations of mouse bone marrow cells in both young adult (8–12-week-old) and old (64–118-week-old) animals on the FVB/n and C57BL/6 backgrounds. In young mice, $p16^{INK4a}$ messenger RNA levels were below detection limits in unfractionated and subpopulations of bone marrow cells. Immunophenotypically defined stem-cell populations—both enriched (Lineage[–]c-Kit⁺Sca1⁺ (Lin[–]Kit⁺Sca1⁺)) and highly enriched (Lin[–]Kit⁺Sca1⁺CD34^{low}Flk-2^{low}) HSC populations^{13,14}—had no detectable levels of $p16^{INK4a}$ mRNA when isolated from young mice (Fig. 1a). However, in old animals increased $p16^{INK4a}$

mRNA was noted in Lin[–]Kit⁺Sca1⁺CD34^{low}Flk-2^{low} cells in both FVB/n (Fig. 1a) and C57BL/6 (data not shown) mice. Unlike $p16^{INK4a}$, expression of *Arf* was detectable in multiple cellular populations in young mice but did not increase significantly in older animals (Supplementary Fig. 1).

To assess the functional role of $p16^{INK4a}$ in these cell populations, we used mice selectively deficient for $p16^{INK4a}$ with intact expression of ARF¹⁵. The absence of $p16^{INK4a}$ was not associated with a compensatory increase in *Arf* expression (Supplementary Fig. 1). Evaluating haematopoietic subpopulations, in young animals we did not observe any differences in the abundance of stem-cell-enriched Lin[–]Kit⁺Sca1⁺ or Lin[–]Kit⁺Sca1⁺CD34^{low}Flk-2^{low} cells, or more mature cells, between wild-type and $p16^{INK4a}$ -deficient ($p16^{INK4a-/-}$) mice (Fig. 1b, c). With advancing age, $p16^{INK4a-/-}$ and wild-type mice showed comparable body size, peripheral blood cell counts and bone marrow cellularity (Supplementary Figs 2 and 3). However, the frequency of long- and short-term HSC-containing populations (Lin[–]Kit⁺Sca1⁺CD34^{low}Flk-2^{low} and Lin[–]Kit⁺Sca1⁺CD34^{low}Flk-2^{low}, respectively) was increased in older $p16^{INK4a-/-}$ mice (Fig. 1c).

$p16^{INK4a}$ expression regulates HSC pool size

Immunophenotypic characterization of haematopoietic stem- and progenitor-cell subsets diverges from function in old animals. It has been noted that the engraftment efficiency of immunophenotypically selected long-term HSCs using SLAM family markers is decreased by approximately threefold in old mice compared with the same population in young mice¹⁶. Other immunophenotypes enriched for stem cells (for example, Lin[–]Thy-1^{low}Sca1⁺c-Kit⁺) give long-term multilineage reconstitution on transplantation at approximately 1 in 5 cells (20%) in young animals¹⁷, whereas cells with the same markers from old animals repopulate with a frequency of only 1 in 78–185 cells

¹Center for Regenerative Medicine, Cancer Center, Massachusetts General Hospital, Harvard Medical School, 185 Cambridge Street, Boston, Massachusetts 02114, USA.

²Harvard Stem Cell Institute, Harvard University, 42 Church Street, Cambridge, Massachusetts 02138, USA. ³Center for Applied Cancer Science and Department of Medical Oncology, Dana-Farber Cancer Institute, Departments of Medicine and Genetics, Harvard Medical School, 44 Binney Street (M413), Boston, Massachusetts 02115, USA.

⁴Departments of Medicine and Genetics, The Lineberger Comprehensive Cancer Center, The University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599-7295, USA. †Present address: Department of Radiation Oncology, University of Pittsburgh School of Medicine, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania 15213, USA.

*These authors contributed equally to this work.

(1.3–0.5%) in old animals^{11,18}. Therefore, functional reconstitution capability declines on a per-cell basis with age.

To assess whether $p16^{\text{INK4a}}$ deficiency affected HSC function, we performed competitive transplants with limiting dilution and analysed peripheral blood at 12 weeks post-transplant. Transplanted 1:1 with wild-type CD45.1^+ competing cells, $p16^{\text{INK4a-/-}}$ CD45.2^+ donor marrow cells from young mice performed comparably, but donor cells from old $p16^{\text{INK4a-/-}}$ mice were superior. Bone marrow from old $p16^{\text{INK4a-/-}}$ animals engrafted and provided a significantly

higher fraction of total peripheral blood cells than did their wild-type littermate counterparts (Supplementary Fig. 4). Limiting-dilution transplants using three dose concentrations of bone marrow cells revealed a higher frequency of multilineage repopulating cells at 12 weeks in $p16^{\text{INK4a-/-}}$ donor bone marrow from old mice (Fig. 1d). This was in direct contrast to young mice, where again no difference was noted between the genotypes. As the total number of mononuclear cells per femur was unchanged between the two genotypes, these data indicate an increase in the absolute number of

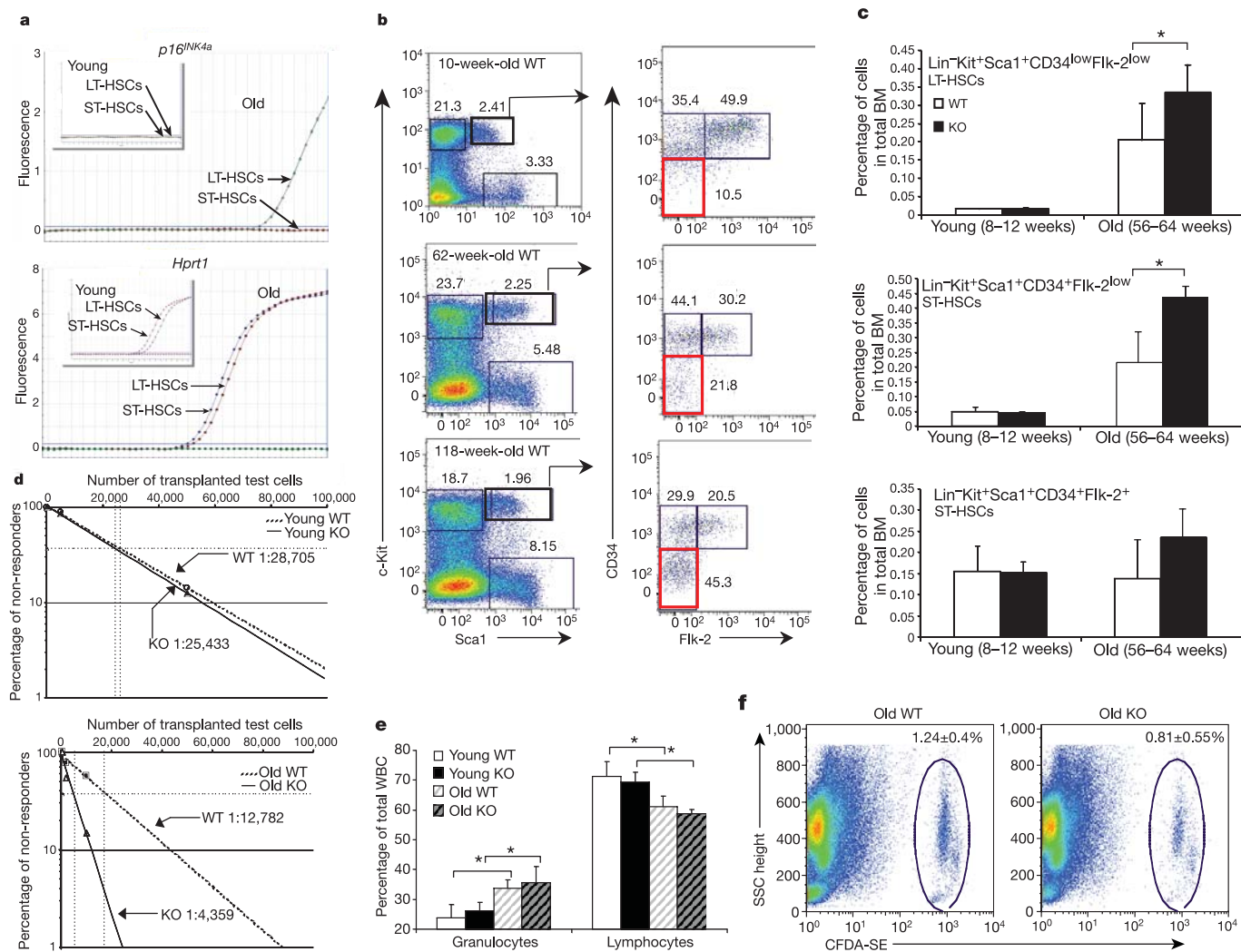


Figure 1 | $p16^{\text{INK4a}}$ expression is upregulated in primitive bone marrow cell populations in aged mice and limits HSC number. **a**, Expression of $p16^{\text{INK4a}}$ within the bone marrow was restricted to the long-term HSC (LT-HSC) subpopulation, as defined by Lin⁻Kit⁺Sca1⁺CD34^{low}Flk-2^{low}, and the Lin⁻Kit⁺Sca1⁺ subpopulation (data not shown) of old wild-type FVB/n (shown here; top panel) and C57BL/6 mice (data not shown). The short-term HSC (ST-HSC) subpopulation, as defined by Lin⁻Kit⁺Sca1⁺CD34⁺Flk-2^{low}, and other subpopulations (data not shown) did not have detectable $p16^{\text{INK4a}}$ expression. $p16^{\text{INK4a}}$ was not detectable in any HSC-containing population of young mice (inset). $Hprt1$ was used as a control (bottom panel); $n = 3$. **b**, The immunophenotypically characterized population of mouse bone marrow cells Lin⁻Kit⁺Sca1⁺CD34^{low}Flk-2^{low}, containing LT-HSCs, were markedly elevated with age. Fluorescence-activated cell sorting (FACS) plots are gated on Lin⁻ bone marrow cells and the numbers indicate the percentage of subpopulations of an individual representative experiment. WT, wild type. **c**, The frequency of long-term (Lin⁻Kit⁺Sca1⁺CD34^{low}Flk-2^{low}) and short-term (Lin⁻Kit⁺Sca1⁺CD34⁺Flk-2^{low} or Lin⁻Kit⁺Sca1⁺CD34⁺Flk-2⁺) HSC-containing populations within the total bone marrow (BM) were comparable between young wild-type (WT) and knockout (KO) mice, but

both populations were increased in aged $p16^{\text{INK4a-/-}}$ mice compared with wild-type littermates. Values are mean $\pm$ s.d. *, $P < 0.05$; $n = 5$. **d**, Limiting-dilution competitive repopulation analysis revealed an elevated functional activity of HSCs with age in the absence of $p16^{\text{INK4a}}$ (bottom panel), whereas no difference between the genotypes in HSC frequency was detected in young animals (top panel). Plotted is the percentage of recipient mice containing less than 1% CD45.2⁺ myeloid and lymphoid blood cells 12 weeks after transplantation. Frequency of HSCs was calculated according to Poisson statistics using L-Calculator software (two-tailed t -test; $P < 0.03$). Ten recipient mice were used for each dose of cells per genotype. This experiment was performed twice with similar results. **e**, $p16^{\text{INK4a}}$ deficiency does not affect myeloid-biased differentiation of aged HSCs on transplantation. Values are mean $\pm$ s.d. *, $P < 0.0002$; $n = 10$. WBC, white blood cells. **f**, Homing ability of carboxyfluorescein diacetate succinimidyl ester (CFDA-SE)-labelled bone marrow cells from aged $p16^{\text{INK4a-/-}}$ animals is reduced compared with wild-type littermate controls. Values are mean $\pm$ s.d. $P < 0.01$; $n = 20$. The experiments were performed in both FVB/n and C57BL/6 backgrounds with similar results. SSC, side scatter.

long-term repopulating cells in older animals deficient in $p16^{INK4a}$.

Age-related changes in stem-cell function such as myeloid-biased differentiation and decreased homing ability have been reported previously^{11,12}. Modifications in these characteristics could potentially affect the interpretation of the repopulation assays described above and therefore were examined. Although we did observe an age-related effect on myeloid versus lymphoid differentiation, the absence of $p16^{INK4a}$ did not have an impact on the differentiation profiles of either blood (Fig. 1e) or bone marrow (Supplementary Fig. 5). In contrast, a $p16^{INK4a}$ -dependent difference was noted in homing capacity. However, contrary to what might be expected to explain the results of the repopulation assays, marrow homing was superior for older wild-type marrow cells compared with age-matched $p16^{INK4a-/-}$ cells (Fig. 1f). Therefore, there was an age-dependent effect of $p16^{INK4a}$ on repopulating ability that did not seem to be associated with $p16^{INK4a}$ -dependent changes in differentiation or improved homing. We conclude that the presence of $p16^{INK4a}$ restricts the number of HSCs.

$p16^{INK4a}$ regulates HSC proliferation and apoptosis

Next, we sought to determine if the absence of $p16^{INK4a}$ had affected cell cycling or apoptosis to account for the change in repopulating cell numbers. In flow cytometric analyses using Hoechst 33342 to assess DNA content, no differences in S/G2/M cell-cycle phases were noted among the various populations of primitive cells (data not shown). Because subtle differences in cell-cycle activity might escape this 'snapshot' detection method, the frequency of cycling cells was quantified by 5-bromodeoxyuridine (BrdU) incorporation. No differences in the fraction of primitive cells in the various subsets

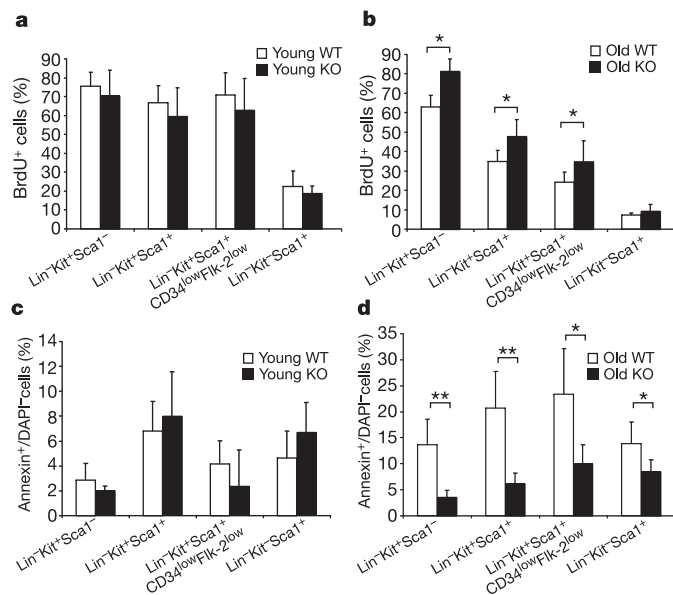


Figure 2 | $p16^{INK4a}$ deficiency results in increased proliferation and reduced frequency of apoptotic events in a transplant setting. Proliferation activity was assessed six weeks after bone marrow transplantation using BrdU incorporation over a 24-h period. **a, b**, No differences in cycling of different subsets of bone marrow cells from young wild-type and knockout mice were detectable (**a**). In contrast, primitive bone marrow cells from old $p16^{INK4a}$ -deficient mice had a significantly higher percentage of cell divisions (**b**). Values are mean $\pm$ s.d. $n = 5$; *, $P < 0.05$. **c, d**, AnnexinV or DAPI (4,6-diamidino-2-phenylindole) staining of bone marrow recipients six weeks post-transplant revealed no difference in the frequency of dying cells in populations isolated from young mice (**c**), but a significant reduction in apoptosis was observed in all primitive populations examined in mice reconstituted with old $p16^{INK4a}$ -deficient bone marrow, relative to age-matched controls (**d**). Values are mean $\pm$ s.d. $n = 5$; *, $P < 0.02$; **, $P < 0.007$.

were detectable between young wild-type and $p16^{INK4a}$ -deficient animals (Supplementary Fig. 6). However, in older animals a trend towards higher BrdU incorporation was observed in the absence of $p16^{INK4a}$ (Supplementary Fig. 7), which was accentuated to a clear difference under stress. Whereas BrdU incorporation in recipients of young wild-type and $p16^{INK4a-/-}$ bone marrow transplants had no significant difference in cycling activity (Fig. 2a), primitive bone marrow cells from older $p16^{INK4a-/-}$ mice showed a significantly higher cycling activity than their wild-type counterparts (Fig. 2b). This change in cell cycling within the knockout genotype is consistent with findings from the neural system, described in an accompanying manuscript¹⁹.

Similarly, no significant differences in the percentage of apoptotic cells in the primitive bone marrow populations were detected between wild-type and $p16^{INK4a-/-}$ animals—in young as well as in old mice—under homeostatic conditions (Supplementary Fig. 8). However, stress again revealed an age-specific change. Apoptotic events in primitive haematopoietic cells from recipients of young $p16^{INK4a-/-}$ and wild-type marrow were not different (Fig. 2c), but old wild-type marrow had a markedly higher apoptotic frequency compared with young marrow (Fig. 2d). The absence of $p16^{INK4a}$ mitigated this effect, reducing the apoptosis frequency closer to that seen in young marrow (Fig. 2d). Therefore, the increase in stem-cell number and function in old knockout animals is associated with a combination of higher cycling activity and a lower frequency of cell death.

$p16^{INK4a}$ regulates the repopulating ability of HSCs

A signature feature of stem cells is their ability to undergo self-renewing cell divisions, an attribute that is crucial for the sustained ability to maintain or repair tissues throughout life. Serial transplantation studies have shown that single clones of bone marrow cells are able to reconstitute lethally irradiated hosts in secondary, tertiary and quaternary transplants over a cumulative period that exceeds the lifespan of the donor, and are used as an experimental measurement of cell-autonomous self-renewing capacity^{20,21}. The serial repopulating or self-renewing capacity of HSCs does decline with age, however²². To address whether $p16^{INK4a}$ affects HSC self-renewal, we performed serial bone marrow transplantation studies with young (8–12-week-old) or old (57–67-week-old) donor mice. We observed that wild-type cells from older donors had a reduced capacity to serially rescue transplanted recipients when compared with younger wild-type donors (Fig. 3a). Comparing young wild-type with young knockout donors, we observed an increase in mortality among those receiving knockout cells. The difference reached a significant level after the third transplantation and was accompanied by decreased colony-forming cell (CFC) frequency in surviving animals (Fig. 3b). Therefore, serial transplantation is associated with premature stem-cell exhaustion in young $p16^{INK4a-/-}$ animals. In contrast, serial bone marrow transplantation using donor bone marrow from older mice demonstrated virtually reciprocal results. The knockout cell recipients showed significantly better survival (Fig. 3a) and superior reconstitution as measured by peripheral blood cell counts for all lineages (Fig. 3b). Consistent with these results, CFC frequency was higher in the knockout recipients at the third transplantation (Fig. 3b). Therefore, $p16^{INK4a}$ has a highly age-dependent effect on HSCs in very select functions, specifically serial repopulation, cell cycling and apoptosis. The specific basis for improved serial transplantation cannot be discerned from these data. Decreased apoptosis alone could affect this phenotype²³, whereas increased cell cycling would only be expected to accomplish this if the cell divisions resulted in self-renewal, a process difficult to show convincingly at the single-cell level *in vivo* and understood at the molecular level only in terms of associated gene expression.

The difference in sequential transplant capability of young versus old $p16^{INK4a-/-}$ animals was notable. The defect in young knockout marrow was unexpected as we did not find $p16^{INK4a}$ expression in young HSCs under homeostatic conditions. However, when we

examined the same cell subsets after transplantation, we did find detectable levels of $p16^{INK4a}$ expression in $\text{Lin}^- \text{Kit}^+ \text{Sca1}^+$ cells (Fig. 3c). Analysis of more-refined subsets was not possible owing to extremely small cell numbers and questionable fidelity of surface markers after transplantation. These findings are consistent with previous reports showing an upregulation of $p16^{INK4a}$ under conditions of cellular stress^{24,25}. Moreover, under the stress of serial bone marrow transplantation, *Arf* expression was also dramatically upregulated in the $\text{Lin}^- \text{Kit}^+ \text{Sca1}^+$ population (Fig. 3d). *Arf* expression has been shown to induce HSC death²⁶. The dual absence of $p16^{INK4a}$ and *Arf*, or *Arf* alone, has been shown not to result in any defect in serial transplantation in young animals²⁷. Therefore, we propose that the deleterious effect of $p16^{INK4a}$ deficiency on young HSCs in serial transplantation is due to increased *Arf* expression and resultant stem-cell death.

$p16^{INK4a}$ suppresses *Hes1* expression in HSCs

The marked improvement in serial repopulating ability with age in the absence of $p16^{INK4a}$ may be due to the documented cycling or apoptosis changes. To indirectly assess whether self-renewal may also participate, levels of self-renewal-associated gene transcripts were analysed at different ages in the wild-type and $p16^{INK4a-/-}$ animals. The polycomb gene *Bmi1* and a downstream effector of Notch activation, *Hes1*, have been experimentally defined to regulate stem cells *in vivo* and were among those evaluated^{26,28}. *Hes1* is of particular interest given that it is known to be sufficient to increase stem-cell preservation *in vitro*, and stem-cell number and function *in vivo*²⁸. No differences in *Bmi1* expression were observed between wild-type

and $p16^{INK4a-/-}$ primitive cells in young and old mice (Fig. 4a), and thus served as a comparison for *Hes1*. *Hes1* expression was not different between young wild-type and $p16^{INK4a-/-}$ mice; however, the $\text{Lin}^- \text{Kit}^+ \text{Sca1}^+$ subpopulation isolated from aged mouse bone marrow showed a significant increase in *Hes1* expression in $p16^{INK4a-/-}$ cells (Fig. 4a). To further explore a link between $p16^{INK4a}$ and *Hes1* expression, we examined mice with a $p16^{INK4a}$ -containing bacterial artificial chromosome (BAC) transgene. These animals have a confirmed increase in $p16^{INK4a}$ expression within bone marrow cells (see the accompanying paper²⁹). In this setting of $p16^{INK4a}$ overexpression, $\text{Lin}^- \text{Kit}^+ \text{Sca1}^+ \text{CD34}^{\text{low}} \text{Flk-2}^{\text{low}}$ cells had decreased *Hes1* expression compared with wild-type littermates (Fig. 4b). Because $p16^{INK4a}$ may affect transcription through binding to Cdk4 and Cdk6, and inhibiting Rb phosphorylation with the consequent suppression of transcriptional activity of E2F, we further explored whether inactivation of Rb could alter *Hes1* expression. The transforming protein E7 of the human papilloma virus (HPV) type 16 binds to the Rb family of proteins de-repressing E2F. We cloned the coding sequence of the HPV16 E7 protein into a murine stem-cell virus (MSCV) plasmid and overexpressed it in a stable transduction of old wild-type $\text{Lin}^- \text{Kit}^+ \text{Sca1}^+$ cells. As controls, we used an empty vector and a mutant E7 lacking the ability to bind Rb (E7($\Delta 21-24$); ref. 30). In three independent experiments, we observed that cells transduced with the MSCV-E7 construct showed an increase in *Hes1* expression compared with the empty vector control. However, no differences in *Bmi1* expression between MSCV-E7 and the vector controls were detected (Fig. 4c). Taken together, these data indicate a role for $p16^{INK4a}$ in regulating the expression of select genes

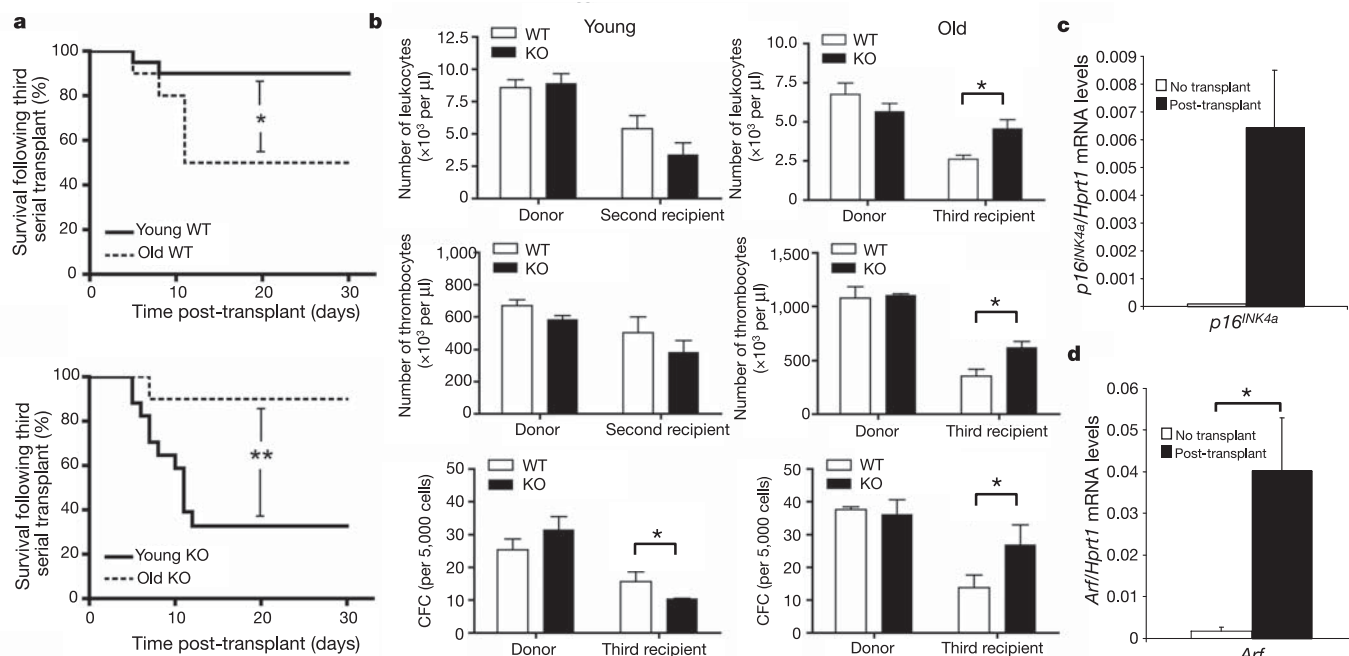


Figure 3 | $p16^{INK4a}$ has an age-dependent effect on stem-cell repopulating ability under the stress of serial bone marrow transplantation. **a**, At the third transplant cycle, recipients of old wild-type bone marrow cells showed a significant reduction in survival relative to young bone marrow cell recipients ($n = 20$; *, $P < 0.03$), indicating an inferior repopulating ability of aged haematopoietic stem cells (top panel). In contrast, recipients of old $p16^{INK4a-/-}$ bone marrow cells (bottom panel) showed a significantly improved survival compared with young $p16^{INK4a-/-}$ bone marrow ($n = 20$; **, $P = 0.0001$). Comparing age-matched bone marrow recipients in the presence or absence of $p16^{INK4a}$ reveals an age-dependent effect of $p16^{INK4a}$ deletion: although young $p16^{INK4a-/-}$ bone marrow cells possess a reduced repopulation ability in sequential bone marrow transplants, aged $p16^{INK4a-/-}$ bone marrow leads to an improvement of repopulating ability (young wild-type or knockout cells, $P < 0.0001$; old wild-type

or knockout cells, $P = 0.02$). **b**, Recipients of a second cycle of $p16^{INK4a-/-}$ bone marrow from young mice (left panels) show a tendency of decreased peripheral blood leukocytes and thrombocytes, and bone marrow cells give rise to fewer CFC colonies than recipients of their wild-type counterpart ($n = 3$; *, $P = 0.01$), whereas recipients of bone marrow from old mice (right panels) show the opposite results with higher peripheral blood cell counts ($n = 10$; *, $P < 0.05$; **, $P < 0.01$) and more colony formation from bone marrow ($n = 10$; **, $P = 0.004$). Values are mean $\pm$ s.d. **c**, **d**, Quantitative PCR analysis for $p16^{INK4a}$ (**c**) and *Arf* (**d**) expression in $\text{Lin}^- \text{Kit}^+ \text{Sca1}^+$ cells of third-round recipients of young bone marrow revealed elevated messenger RNA levels compared with untransplanted control cells ($n = 4$; *, $P < 0.02$; **, $P < 0.004$). Values are mean $\pm$ s.d.

associated with stem-cell self-renewal. In particular, *Hes1* expression is affected by $p16^{INK4a}$ and the $p16^{INK4a}$ target Rb, and though we cannot definitively conclude that the *Hes1* expression changes caused the alteration in stem-cell phenotype, two issues are worthy of mention. First, the previously defined ability of *Hes1* expression to increase HSC number *in vivo* and, second, increased Notch signalling has been associated with an improvement in aged muscle regeneration by satellite cells in reports by others³¹. These data indicate that the Notch pathway may represent a common theme

for altering ageing stem-cell function and tissue regeneration—a hypothesis that merits further investigation.

Conclusions

Stem-cell changes with age were not uniformly modified by the absence of $p16^{INK4a}$, but crucial functions relevant for persistence and survival under stress were (Fig. 4d). Down-modulating $p16^{INK4a}$ decreased age-related stem-cell apoptosis with stress and improved repeated repopulation of injured tissue. The model of bone marrow transplantation used here directly tests stem-cell-autonomous tissue regeneration after injury. Modifying specific age-related functional changes in stem cells through alteration of $p16^{INK4a}$ status can improve tissue regeneration and animal survival after injury. Focusing on the stem cell and its molecular regulators may provide an opportunity for altering some tissue and organismal consequences of age.

METHODS

Mice. FVB/n and C57BL/6 wild-type and $p16^{INK4a-/-}$ mice were bred in-house in a pathogen-free environment. The $p16^{INK4a}$ knockout mouse was generated on an FVB/n background as described previously¹⁵ and backcrossed to C57BL/6 for eight generations. FVB/n animals were used for initial characterization; however, results were confirmed in the C57BL/6 background. The C57BL/6 background was used preferentially owing to the availability of an antibody-based detection method of donor cells by the CD45 locus polymorphism. The design and validation of $p16^{INK4a}$ BAC transgenic mice is described in an accompanying paper²⁹. The experimental cohort of transgenic mice and littermate controls was generated after backcrossing two generations to C57BL/6, and was housed and aged in the University of North Carolina barrier facility on a standard diet. The Institutional Animal Care and Use Committee of the University of North Carolina and the Subcommittee on Research Animal Care of the Massachusetts General Hospital approved all animal work according to federal and institutional policies and regulations.

Retroviral gene transfer of $Lin^{-}Kit^{+}Sca1^{+}$ cells. Complementary DNAs encoding HPV16 E7 and E7($\Delta 21-24$) sequence were a gift from K. Munger³⁰ and were subcloned into the retroviral vector MSCV. Virus production and transduction of sorted $Lin^{-}Kit^{+}Sca1^{+}$ cells was performed as described previously³². Two days after virus transduction, cells were sorted for GFP-positive cells and cultured for a further eight days in HSC medium with subsequent RNA isolation and gene expression analysis.

Cells and cell culture. Bone marrow was harvested as described previously¹ and grown in colony-forming unit in culture (CFU-C) assays according to the manufacturer's protocols (Stem Cell Technologies). Sorted $Lin^{-}Kit^{+}Sca1^{+}$ cells were cultured in HSC medium: X-Vivo 15 (Cambrex) supplemented with 10% detoxified BSA (Stem Cell Technologies), 100 U ml⁻¹ penicillin (BioWhittaker), 100 U ml⁻¹ streptomycin (Cellgro), 2 mM L-glutamine (BioWhittaker) and 0.1 mM 2-mercaptoethanol (Sigma). Before virus transduction, $Lin^{-}Kit^{+}Sca1^{+}$ cells were cultured in the presence of 50 ng ml⁻¹ recombinant murine (rm) SCF, 50 ng ml⁻¹ rmTPO, 50 ng ml⁻¹ rmFlt-3L and 20 ng ml⁻¹ rmIL3 (all from PeproTech). Twenty-four hours after virus transduction, cells were maintained in fresh HSC medium in the presence of 10 ng ml⁻¹ rmSCF and 10 ng ml⁻¹ rmTPO.

Flow cytometric analysis and sorting of subpopulations. Lineage staining used a cocktail of biotinylated anti-mouse antibodies to Mac-1 α (CD11b), Gr-1 (Ly-6G and Ly-6C), Ter119 (Ly-76), CD3 ϵ , CD4, CD8 α (Ly-2) and B220 (CD45R) (BD Biosciences). For detection and sorting, we used streptavidin conjugated with phycoerythrin (PE) or Cy7, c-Kit-allophycocyanin (APC) (CD117), Flk-2-PE (CD135), CD34-fluorescein isothiocyanate (FITC) (all from BD Biosciences) and Sca1-PE/Cy5.5 (Ly-6A/E, Caltag). For BrdU incorporation, we used the APC-BrdU Flow Kit (BD Biosciences) after a single intraperitoneal injection of BrdU (1 mg per 6 g of body weight) and admixture of 1 mg ml⁻¹ of BrdU (Sigma) to the drinking water for approximately 20 h. Surface staining for lineage markers was performed as above using Sca1-PE/Cy5.5, c-Kit-APC/Cy7 (eBioscience) and CD34-FITC. For the apoptosis assay, we used DAPI (4,6-diamidino-2-phenylindole; Molecular Probes) and annexinV-APC (BD Biosciences).

Transplantation assays. For serial transplantation, 4×10^6 whole-bone-marrow cells from either 8–12- or 52–67-week-old male wild-type or $p16^{INK4a}$ knockout littermates were injected into lethally irradiated (10 Gy) female recipient mice. Serial transplants were performed in both FVB/n and C57BL/6 mice strains. Engraftment efficiency in FVB/n recipients was monitored by Y-chromosome polymerase chain reaction (PCR) for donor contribution. In C57BL/6 mice, transplants were performed into the congenic CD45.1⁺ B6.SJL mouse strain and

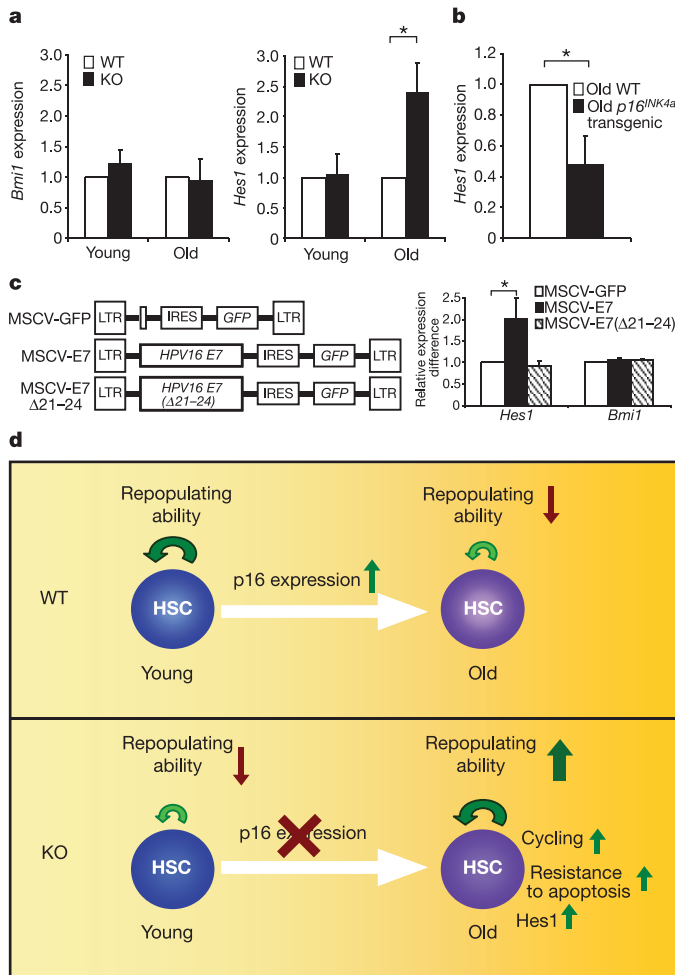


Figure 4 | $p16^{INK4a}$ regulates expression of the self-renewal-associated gene *Hes1*. **a**, Quantitative PCR analyses in sorted $Lin^{-}Kit^{+}Sca1^{+}$ population of mouse bone marrow revealed no differences in expression levels of *Bmi1* and *Hes1* in young mice, but showed significantly higher levels of *Hes1* in old $p16^{INK4a-/-}$ cells compared with their wild-type littermates. Values are presented as relative expression differences between wild-type and knockout cells as mean $\pm$ s.d. of three experiments; *, $P < 0.05$. **b**, Sixty-three-week-old $p16^{INK4a-/-}$ BAC transgenic mice showed a reduction in *Hes1* expression in sorted $Lin^{-}Kit^{+}Sca1^{+}CD34^{low}Flk2^{low}$ populations of bone marrow compared with wild-type littermates ($n = 2$; *, $P < 0.05$; error bars represent s.d.). **c**, Expression of HPV16 E7 protein in sorted old wild-type $Lin^{-}Kit^{+}Sca1^{+}$ cells resulted in higher levels of *Hes1* mRNA compared with the control cells, whereas *Bmi1* expression remained unchanged. Data are presented as changes of relative expression normalized to *Hprt1* and values are mean $\pm$ s.d. $n = 3$; *, $P < 0.005$. **d**, Proposed model of the role of $p16^{INK4a}$ in the regulation of the repopulation ability of haematopoietic stem cells under proliferative stress. With advanced age and increased $p16^{INK4a}$ expression, the repopulating ability on a per-cell basis is dramatically decreased (top panel). In the absence of $p16^{INK4a}$ in young mice, the repopulating ability is diminished; however, in aged animals $p16^{INK4a}$ deletion is accompanied by superior repopulating ability compared with the wild-type counterpart.

monitored for CD45.2⁺ donor-derived cells. Peripheral blood cell counts were obtained by tail vein nicking 6 and 12 weeks post-transplantation. Six weeks post-transplantation, recipients were used as donors for the next transplantation cycle and for *in vitro* assays. Transplants were discontinued when survival was below 50%.

For the competitive repopulation unit (CRU) assay with bone marrow from young mice, we used 5×10^3 , 5×10^4 and 5×10^5 wild-type or knockout whole-bone-marrow cells from CD45.2 littermates (eight weeks old) mixed with 5×10^5 CD45.1 (competitor) wild-type cells and injected into lethally irradiated CD45.1 recipient mice. For the CRU assay with old bone marrow, we used 8×10^3 , 4×10^4 , 2×10^5 and 1×10^5 wild-type or knockout whole-bone-marrow cells from CD45.2 littermates (64 weeks old) mixed with 2×10^5 CD45.1 (competitor) wild-type cells and injected into lethally irradiated CD45.1 recipient mice. Repopulation was assessed by flow cytometry at weeks 6 and 12 post-transplant for multilineage reconstitution. Calculation of CRUs in limiting-dilution experiments was conducted using L-Calc software (StemCell Technologies).

To assess homing *in vivo*, bone marrow cells were labelled with 5 mM carboxyfluorescein diacetate succinimidyl ester (CFDA-SE; Molecular Probes) in accordance with the manufacturer's instructions and analysed as described previously³³.

PCR and gene expression analyses. *p16^{INK4a}* genotyping was performed as described¹⁵, and Y-chromosome PCR also as described¹. RNA was isolated from fluorescence-activated cell sorting (FACS) bone marrow subpopulations using the PicoPure Kit (Arcturus Bioscience). Pre-designed PCR assays for *Hprt1*, *Bmi1*, *Gfi1* and *Hes1* were purchased from Applied Biosystems (assay numbers Mm00446968, Mm00776122, Mm00515853 and Mm00468601, respectively). Primers and probes for *p16^{INK4a}* and *Arf* were used as described previously⁶.

Statistical analysis. The Mann-Whitney test was used to determine the significance of the difference between the means.

Received 13 April; accepted 7 August 2006.

Published online 6 September 2006.

- Cheng, T. *et al.* Hematopoietic stem cell quiescence maintained by p21cip1/waf1. *Science* **287**, 1804–1808 (2000).
- Walkley, C. R., Fero, M. L., Chien, W. M., Purton, L. E. & McArthur, G. A. Negative cell-cycle regulators cooperatively control self-renewal and differentiation of haematopoietic stem cells. *Nature Cell Biol.* **7**, 172–178 (2005).
- Yuan, Y., Shen, H., Franklin, D. S., Scadden, D. T. & Cheng, T. *In vivo* self-renewing divisions of haematopoietic stem cells are increased in the absence of the early G1-phase inhibitor, p18^{INK4C}. *Nature Cell Biol.* **6**, 436–442 (2004).
- Sharpless, N. E. *Ink4a/Arf* links senescence and aging. *Exp. Gerontol.* **39**, 1751–1759 (2004).
- Rocco, J. W. & Sidransky, D. p16(MTS-1/CDKN2/INK4a) in cancer progression. *Exp. Cell Res.* **264**, 42–55 (2001).
- Krishnamurthy, J. *et al.* *Ink4a/Arf* expression is a biomarker of aging. *J. Clin. Invest.* **114**, 1299–1307 (2004).
- Zindy, F., Quelle, D. E., Roussel, M. F. & Sherr, C. J. Expression of the p16^{INK4a} tumour suppressor versus other INK4 family members during mouse development and aging. *Oncogene* **15**, 203–211 (1997).
- Campisi, J. Cellular senescence as a tumour-suppressor mechanism. *Trends Cell Biol.* **11**, S27–S31 (2001).
- Chen, J., Astle, C. M. & Harrison, D. E. Development and aging of primitive hematopoietic stem cells in BALB/cBy mice. *Exp. Hematol.* **27**, 928–935 (1999).
- Ogden, D. A. & Micklem, H. S. The fate of serially transplanted bone marrow cell populations from young and old donors. *Transplantation* **22**, 287–293 (1976).
- Morrison, S. J., Wandycz, A. M., Akashi, K., Globerson, A. & Weissman, I. L. The aging of hematopoietic stem cells. *Nature Med.* **2**, 1011–1016 (1996).
- Liang, Y., Van Zant, G. & Szilvassy, S. J. Effects of aging on the homing and engraftment of murine hematopoietic stem and progenitor cells. *Blood* **106**, 1479–1487 (2005).
- Osawa, M., Hanada, K., Hamada, H. & Nakauchi, H. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* **273**, 242–245 (1996).
- Adolfsson, J. *et al.* Identification of Flt3⁺ lympho-myeloid stem cells lacking erythro-megakaryocytic potential. A revised road map for adult blood lineage commitment. *Cell* **121**, 295–306 (2005).
- Sharpless, N. E. *et al.* Loss of p16^{INK4a} with retention of p19^{Arf} predisposes mice to tumorigenesis. *Nature* **413**, 86–91 (2001).
- Yilmaz, O. H., Kiel, M. J. & Morrison, S. J. SLAM family markers are conserved among hematopoietic stem cells from old and reconstituted mice and markedly increase their purity. *Blood* **107**, 924–930 (2006).
- Wagers, A. J., Sherwood, R. L., Christensen, J. L. & Weissman, I. L. Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science* **297**, 2256–2259 (2002).
- Morrison, S. J., Wright, D. E. & Weissman, I. L. Cyclophosphamide/granulocyte colony-stimulating factor induces hematopoietic stem cells to proliferate prior to mobilization. *Proc. Natl Acad. Sci. USA* **94**, 1908–1913 (1997).
- Molofsky, A. V. *et al.* Increasing p16^{INK4a} expression decreases forebrain progenitors and neurogenesis during ageing. *Nature* advance online publication, doi:10.1038/nature05091 (6 September 2006).
- Siminovich, L., Till, J. E. & McCulloch, E. A. Decline in colony-forming ability of marrow cells subjected to serial transplantation into irradiated mice. *J. Cell. Physiol.* **64**, 23–31 (1964).
- Harrison, D. E. Normal function of transplanted mouse erythrocyte precursors for 21 months beyond donor life spans. *Nature New Biol.* **237**, 220–222 (1972).
- Chen, J., Astle, C. M. & Harrison, D. E. Genetic regulation of primitive hematopoietic stem cell senescence. *Exp. Hematol.* **28**, 442–450 (2000).
- Domen, J., Cheshier, S. H. & Weissman, I. L. The role of apoptosis in the regulation of hematopoietic stem cells: overexpression of BCL-2 increases both their number and repopulation potential. *J. Exp. Med.* **191**, 253–264 (2000).
- Chkhotua, A. B. *et al.* Increased expression of p16^{INK4a} and p27^{Kip1} cyclin-dependent kinase inhibitor genes in aging human kidney and chronic allograft nephropathy. *Am. J. Kidney Dis.* **41**, 1303–1313 (2003).
- Chimenti, C. *et al.* Senescence and death of primitive cells and myocytes lead to premature cardiac aging and heart failure. *Circ. Res.* **93**, 604–613 (2003).
- Park, I. K. *et al.* Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature* **423**, 302–305 (2003).
- Stepanova, L. & Sorrentino, B. P. A limited role for p16^{INK4a} and p19^{Arf} in the loss of hematopoietic stem cells during proliferative stress. *Blood* **106**, 827–832 (2005).
- Kuniso, A. *et al.* HES-1 preserves purified hematopoietic stem cells *ex vivo* and accumulates side population cells *in vivo*. *Blood* **101**, 1777–1783 (2003).
- Krishnamurthy, J. *et al.* p16^{INK4a} induces an age-dependent decline in islet regenerative potential. *Nature* advance online publication, doi:10.1038/nature05092 (6 September 2006).
- Phelps, W. C., Munger, K., Yee, C. L., Barnes, J. A. & Howley, P. M. Structure–function analysis of the human papilloma virus type 16 E7 oncoprotein. *J. Virol.* **66**, 2418–2427 (1992).
- Conboy, I. M. *et al.* Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* **433**, 760–764 (2005).
- Stier, S., Cheng, T., Dombkowski, D., Carlesso, N. & Scadden, D. T. Notch1 activation increases hematopoietic stem cell self-renewal *in vivo* and favors lymphoid over myeloid lineage outcome. *Blood* **99**, 2369–2378 (2002).
- Adams, G. B. *et al.* Stem cell engraftment at the endosteal niche is specified by the calcium-sensing receptor. *Nature* **439**, 599–603 (2006).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank K. Munger for the HPV16 E7 clones. We also thank the National Institutes of Health (D.T.S., H.E.F., Y.S., T.C., R.A.D. and N.E.S.), Dr. Mildred Scheel Stiftung fuer Krebsforschung (V.J.), Deutsche Forschungsgemeinschaft (R.F.), The Ellison Medical Foundation and American Cancer Society (R.A.D.), The Paul Beeson Program in Aging Research (N.E.S.), The Sidney Kimmel Foundation (N.E.S.) and The Burroughs Wellcome Foundation (D.T.S.).

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to D.T.S. (scadden.david@mgh.harvard.edu).

Infall of gas as the formation mechanism of stars up to 20 times more massive than the Sun

Maria T. Beltrán¹, Riccardo Cesaroni², Claudio Codella³, Leonardo Testi², Ray S. Furuya⁴ & Luca Olmi³

Theory predicts and observations confirm that low-mass stars (like the Sun) in their early life grow by accreting gas from the surrounding material. But for stars ~ 10 times more massive than the Sun ($\sim 10M_{\odot}$), the powerful stellar radiation is expected to inhibit accretion¹ and thus limit the growth of their mass. Clearly, stars with masses $>10M_{\odot}$ exist, so there must be a way for them to form. The problem may be solved by non-spherical accretion^{2,3}, which allows some of the stellar photons to escape along the symmetry axis where the density is lower. The recent detection of rotating disks^{4–6} and toroids⁷ around very young massive stars has lent support to the idea that high-mass ($\geq 8M_{\odot}$) stars could form in this way. Here we report observations of an ammonia line towards a high-mass star forming region. We conclude that the gas is falling inwards towards a very young star of $\sim 20M_{\odot}$, in line with theoretical predictions of non-spherical accretion.

In the work we report here, we have revealed the simultaneous presence of three elements in the same massive object: outflow, rotation and infall. Although evidence of circumstellar disks or toroids in massive objects had been provided by a number of observational studies^{4–10} (see ref. 11 for a review), it remained to be proved that these are accretion structures, sustaining the growth of the central star(s). Evidence of infall had been found only in a very limited number of massive objects^{12–17}, but in no case had the simultaneous presence of rotation, outflow and infall towards an O-B (proto)star been established. Our findings hence represent a substantial advance in this field.

The source under investigation is G24.78+0.08, a massive star forming region located at a distance of 7.7 kpc, studied by us in great detail^{7,18–21}. This contains a cluster of young stellar objects, three of them associated with rotating toroids. In one of these, G24 A1, the presence of an early-type star is witnessed by a hypercompact H II region (M.T.B. *et al.*, manuscript in preparation) with a diameter $\leq 0.2''$ ($\leq 1,500$ AU) and located at the geometrical centre of the toroid (see Fig. 1). The continuum spectrum resembles that of a classical (Strömgren) H II region around a zero-age main sequence star of spectral type O9.5 (ref. 18), corresponding to a mass of $\sim 20M_{\odot}$, a luminosity of $\sim 33,000L_{\odot}$ (where $L_{\odot}$ is the solar luminosity) and a Lyman continuum of $5 \times 10^{47} \text{ s}^{-1}$. As the H II region is very bright at centimetre wavelengths ($\geq 2,000$ K), it is easy to observe the colder molecular gas (~ 100 K) in absorption against it. If the toroid is not only rotating, but also accreting onto the central star, one expects to see absorption at positive velocities (that is, red-shifted by Doppler effect) relative to the stellar velocity^{12–15}. The latter ($\sim 110.8 \text{ km s}^{-1}$) is equal to the mean velocity of the gas in the toroid, estimated from molecules whose lines are not affected by absorption²¹, such as methyl cyanide (CH_3CN).

With this in mind, we observed simultaneously the continuum

emission and the ammonia (NH_3)(2,2) inversion transition at 1.3-cm wavelength using the Very Large Array (VLA) interferometer of the National Radio Astronomy Observatory (NRAO) in the B configuration. As expected, the line is seen in absorption towards the continuum of the H II region: this is shown by the white contours in Fig. 1a, where one can also see the emission (black contours) detected towards the adjacent toroid G24 A2, not discussed here. Clearly, the peak of absorption as well as the peak of the 1.3-cm continuum emission (that is, of the H II region) lie at the centre of the toroid previously imaged by us (yellow line) and along the axis of the associated bipolar outflow (black arrows). The velocity gradient in the rotating toroid is shown in Fig. 1b, overlaid on an image and contour map of the CH_3CN emission at 1.4-mm wavelength previously obtained by us^{7,21} with the IRAM Plateau de Bure interferometer (PdBI) in the most extended configuration.

Figure 2 illustrates the most important finding of our study. In order to understand this figure, one must keep in mind that the NH_3 inversion transitions have a complex structure, made of a 'main' component and four 'hyperfine satellites'. The former line is intrinsically stronger than the latter ones by a factor of ~ 14 , so that, roughly speaking, emission in the main line is representative of lower gas densities, whereas the satellites trace the densest material. In Fig. 2 we show position–velocity plots for both the main line (Fig. 2a) and the mean satellite emission (Fig. 2b), where the offset in position is measured along the plane of the rotating toroid. In Fig. 2c we show the same plot for the $\text{CH}_3\text{CN}(12-11) K=3$ line, which was used by us to trace rotation in the toroid⁷. Three considerations are in order: (1) towards the hypercompact H II region, the satellite absorption is strongly biased towards positive velocities, that is, red-shifted with respect to the velocity of the star; (2) the NH_3 main line is seen in absorption at both positive and negative velocities, but the blue-shifted absorption is fainter, broader, and more optically thin than the red-shifted one; and (3) the velocity gradient seen in the CH_3CN line (indicated by the green line in Fig. 2c) is detected also in the NH_3 main line (green line across the two emission peaks in Fig. 2a), thus confirming the presence of rotation.

The important conclusion is that the densest gas seen in the satellite absorption is moving away from the observer at a speed of $\sim 2 \text{ km s}^{-1}$ (see Fig. 2b) towards the hypercompact H II region, namely towards the star. This fact proves the presence of infall onto the O-type star at the centre of the toroid. On the other hand, the faint and broad blue-shifted absorption seen in the main line is probably due to the low-column-density gas in the lobe of the molecular outflow that is moving towards the observer: this is indicated by the lower main line/satellite ratio (that is, lower optical depth) observed in the blue line wing with respect to the one in the red-shifted absorption. Incidentally, we note that the broad blue-shifted absorption can be used to discriminate between sources G24

¹Departament d'Astronomia i Meteorologia, Universitat de Barcelona, Av. Diagonal, 647, 08028, Barcelona, Catalunya, Spain. ²Osservatorio Astrofisico di Arcetri, ³Istituto di Radioastronomia, INAF, Sezione di Firenze, Largo E. Fermi 5, 50125 Firenze, Italy. ⁴Subaru Telescope, National Astronomical Observatory of Japan, 650 North Aohoku Place, Hilo, Hawaii 96720, USA.

A1 and G24 A2 as the origin of the outflow. As the blue-shifted lobe is directed towards the northwest¹⁹, it cannot originate from G24 A2 or it would not pass in front of G24 A1. We hence believe that the star in G24 A1 is powering the flow.

What are the implications of the detection of infall in G24 A1? Using the properties of the $\text{NH}_3(2,2)$ transition and an estimate of the gas temperature²¹, one can derive the column density of the infalling gas, N_{H_2} , from the ratio between the main line intensity and that of the satellites²², assuming an NH_3 abundance relative to H_2 of 10^{-6} – 10^{-7} (refs 18, 23). From N_{H_2} and the infall velocity, $v_{\text{inf}} \approx 2 \text{ km s}^{-1}$, assuming free-fall one obtains the mass accretion rate onto the star inside a solid angle Ω : $\dot{M}_{\text{acc}} \approx \Omega/(4\pi)[4 \times 10^{-4} - 10^{-2}] M_{\odot} \text{ yr}^{-1}$.

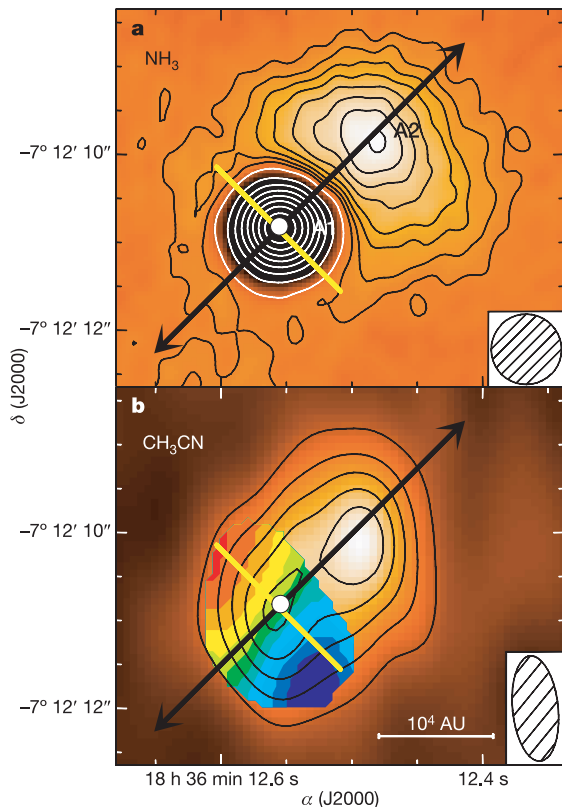


Figure 1 | Absorption and emission by molecular gas towards the hypercompact H II region G24 A1. **a**, Map of the intensity integrated under the $\text{NH}_3(2,2)$ inversion line (colour image). Black contours indicate positive intensity (emission), while white contours are negative (absorption). The white filled circle marks the position of the hypercompact H II region detected in the continuum emission at 1.3-cm wavelength¹⁷ (M. T. B. *et al.*, manuscript in preparation). The black arrows outline the direction of the bipolar outflow, ejected along the axis of the disk. The disk plane is denoted by the yellow line. Note the deep absorption towards the H II region, at the centre of the toroid. The final maps have been reconstructed with a circular synthesized beam of $0.8'' \times 0.8''$, which is represented by the hatched circle at bottom right. The 1σ noise of the integrated $\text{NH}_3(2,2)$ line is $0.6 \text{ mJy beam}^{-1}$. Contour levels start at a $\pm 3\sigma$ level and vary by 3σ (positive contours) and 12σ (negative contours). **b**, Map (image and contours) of the intensity integrated under the $\text{CH}_3\text{CN}(12-11) K=3$ line²¹ outlining the toroids in G24 A1 and G24 A2 (the latter is not discussed in this Letter). The colour scale corresponds to the velocity field of the toroid in G24 A1: note how the velocity changes gradually from red-shifted to blue-shifted, consistent with rotation about the outflow axis. A velocity gradient, similar to that in G24 A1, has also been detected⁷ in the other core G24 A2, but is neither shown nor discussed here. Symbols have the same meaning as above. The resulting synthesized beam is $1.2'' \times 0.5''$ at position angle $\text{PA} = -174^\circ$ and is represented by the hatched ellipse at the bottom right. The 1σ noise of the intensity integrated under the $\text{CH}_3\text{CN}(12-11) K=3$ line is 35 mJy beam^{-1} . Contour levels start at $0.25 \text{ Jy beam}^{-1}$ and increase by 0.2 Jy beam^{-1} . δ , declination; α , right ascension.

A detailed derivation of the mass accretion rate is presented in Supplementary Information. The range of values reflects the uncertainty on N_{H_2} and on the radius at which v_{inf} is measured. Noticeably, $\dot{M}_{\text{acc}}$ is much larger than the critical rate above which formation of an H II region is inhibited²⁴ if accretion is spherically symmetric (that is, for $\Omega = 4\pi$). For an O9.5 star this is $\dot{M}_{\text{inh}} \approx 8 \times 10^{-6} M_{\odot} \text{ yr}^{-1}$, much less than $\dot{M}_{\text{acc}}$: this should suffice to prevent the formation of an H II region. The fact that, instead, an H II region is detected can be explained only if the accretion is not spherically symmetric ($\Omega < 4\pi$), which in turn supports the existence of a circumstellar disk-like structure, detected by us on a larger scale as a toroid.

It is also interesting to estimate the rate needed for the ram pressure to overcome the thermal pressure of the H II region ($\dot{M}_{\text{HII}} \approx \Omega/(4\pi) 2 \times 10^{-4} M_{\odot} \text{ yr}^{-1}$). This has been obtained assuming an electron density and diameter of the H II region respectively of $\sim 10^6 \text{ cm}^{-3}$ (ref. 18) and $0.2''$ (see above) or 0.0075 pc . One sees that $\dot{M}_{\text{acc}} \geq \dot{M}_{\text{HII}}$ independently of Ω , which indicates that the accretion is large enough to brake or slow down the expansion of the H II region through the toroid. This conclusion is strengthened if one takes into account the effect of stellar gravity, which is probably non-negligible

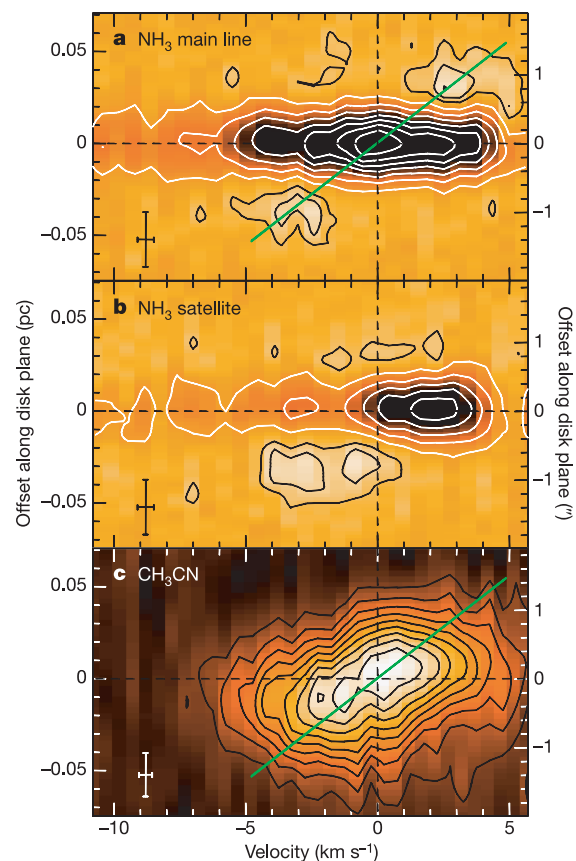


Figure 2 | Velocity field in the massive toroid G24 A1. **a**, Position-velocity plot of the $\text{NH}_3(2,2)$ main line intensity. The positional cut is made along the plane of the disk, $\text{PA} = -135^\circ$, that is, along the yellow line in Fig. 1. Both offset and velocity are computed with respect to the star. Black and white contours correspond respectively to emission and absorption. The continuum has been subtracted from the line emission. The 1σ noise is 1 mJy beam^{-1} . Contour levels start at a 3σ level and increase by 3σ (positive contours) and 6σ (negative contours). The error bars denote the angular and spectral resolution. The original spectral resolution was $\sim 0.31 \text{ km s}^{-1}$, which was degraded by a factor of 2 to improve the signal-to-noise ratio by averaging pairs of adjacent channels. Note the velocity gradient shown by the green line. **b**, As **a** but for the $\text{NH}_3(2,2)$ satellite line. Note how the absorption peak is red-shifted with respect to the stellar velocity. **c**, As **a** but for the $\text{CH}_3\text{CN}(12-11) K=3$ line: the velocity gradient indicated by the green line is indicative of rotation²¹. The 1σ noise is 48 mJy beam^{-1} .

in such a small H II region. Detailed models support this conclusion, and predict that hypercompact H II regions could be long-lived because of trapping of the ionized gas²⁵ owing to infall in the gravitational field of the star.

We now consider the effect of our findings on current theories of massive star formation. Stars as massive as $\sim 8M_{\odot}$ are believed to reach the zero-age main sequence still accreting. At this stage, their powerful radiation pressure appears to be strong enough to halt the infalling material^{1,26}, which should inhibit further growth of the star beyond the limit of $\sim 8M_{\odot}$. In order to solve this problem, merging of low-mass stars has been proposed²⁷, although—even in the most favourable case of induced binary mergers—to be effective this scenario requires very large stellar densities, at least of the order of $10^6 \text{ stars pc}^{-3}$. Increasing the mass accretion rate may be a viable theoretical solution to overcome the radiation pressure with the ram pressure of the infalling material. Moreover, this would solve the lifetime problem²⁸; this is related to the fact that with accretion rates typical of low-mass stars ($10^{-5} M_{\odot} \text{ yr}^{-1}$) the time needed to form a star $> 10M_{\odot}$ would be too long ($> 10^6 \text{ yr}$), comparable to the entire lifetime of the star. Higher rates can be achieved either by assuming a large turbulent pressure^{28,29} in the molecular cores forming O-B type stars, or deepening the gravitational well³⁰ around a massive star by surrounding it with a tight cluster of lower mass companions. Non-spherical accretion may also soften the problem^{2,3}: focusing accretion into a circumstellar disk reduces significantly the radiation pressure force along the equator by beaming the radiation in the direction of the poles.

In our case, direct accretion onto the central massive (proto-)star has not yet been detected, so that the system may be in a (relatively) late phase, when the final mass of the central object has been assembled and the infalling material is being deflected in the outflow instead of being accreted. Nevertheless, our detection of infall towards G24 A1 is an important milestone towards an observational constraint of the theoretical models. In fact, even if the accretion phase were finished, the presence of a rotating toroid infalling on a hypercompact H II region, located at the base of a powerful collimated bipolar flow, is broadly in agreement with the expectations of the non-spherical accretion scenario for the formation of massive stars. Our results thus suggest that this is a plausible mechanism for the formation of stars at least as massive as $20M_{\odot}$. More ‘exotic’ mechanisms (such as stellar mergers) might still be required to form stars of even higher mass.

Received 12 April; accepted 3 July 2006.

1. Kahn, F. D. C cocoons around early-type stars. *Astron. Astrophys.* **37**, 149–162 (1974).
2. Yorke, H. W. & Sonnhalter, C. On the formation of massive stars. *Astrophys. J.* **569**, 846–862 (2002).
3. Krumholz, M. R., McKee, C. F. & Klein, R. I. How protostellar outflows help massive stars form. *Astrophys. J.* **618**, L33–L36 (2005).
4. Cesaroni, R. *et al.* A study of the Keplerian accretion disk and precessing outflow in the massive protostar IRAS 20126+4104. *Astron. Astrophys.* **434**, 1039–1054 (2005).
5. Chini, R. *et al.* The formation of a massive protostar through the disk accretion of gas. *Nature* **429**, 155–157 (2004).
6. Patel, N. *et al.* A disk of dust and molecular gas around a high-mass protostar. *Nature* **437**, 109–111 (2005).

7. Beltrán, M. T. *et al.* Rotating disks in high-mass young stellar objects. *Astrophys. J.* **601**, L187–L190 (2004).
8. Cesaroni, R., Felli, M., Testi, L., Walmsley, C. M. & Olmi, L. The disk-outflow system around the high-mass (proto)star IRAS 20126+4104. *Astron. Astrophys.* **325**, 725–744 (1997).
9. Jiang, Z. *et al.* A circumstellar disk associated with a massive protostellar object. *Nature* **437**, 112–115 (2005).
10. Cesaroni, R. in *Massive Star Birth: A Crossroads of Astrophysics* (eds Cesaroni, R., Felli, M., Churchwell, E. & Walmsley, M.) 59–69 (Proc. IAU Symp. 227, Cambridge Univ. Press, Cambridge, UK, 2005).
11. Cesaroni, R. Outflow, infall, and rotation in high-mass star forming regions. *Astrophys. Space Sci.* **295**, 5–17 (2005).
12. Ho, P. T. P. & Haschick, A. D. Molecular clouds associated with compact H II regions. III. Spin-up and collapse in the core of G10.6–0.4. *Astrophys. J.* **304**, 501–514 (1986).
13. Keto, E. R., Ho, P. T. P. & Haschick, A. D. Temperature and density structure of the collapsing core of G10.6–0.4. *Astrophys. J.* **318**, 712–728 (1987).
14. Zhang, Q. & Ho, P. T. P. Dynamical collapse in W51 massive cores: NH₃ observations. *Astrophys. J.* **488**, 241–257 (1997).
15. Hofner, P., Peterson, S. & Cesaroni, R. Ammonia absorption toward the ultracompact H II regions G45.12+0.13 and G45.47+0.05. *Astrophys. J.* **514**, 899–908 (1999).
16. Sollins, P. & Ho, P. T. P. The molecular accretion flow in G10.6–0.4. *Astrophys. J.* **630**, 987–995 (2005).
17. Zhang, Q. in *Massive Star Birth: A Crossroads of Astrophysics* (eds Cesaroni, R., Felli, M., Churchwell, E. & Walmsley, M.) 135–144 (Proc. IAU Symp. 227, Cambridge Univ. Press, Cambridge, UK, 2005).
18. Codella, C., Testi, L. & Cesaroni, R. The molecular environment of H₂O masers: VLA ammonia observations. *Astron. Astrophys.* **325**, 282–294 (1997).
19. Furuya, R. S. *et al.* G24.78+0.08: A cluster of high-mass (proto)stars. *Astron. Astrophys.* **390**, L1–L4 (2002).
20. Cesaroni, R., Codella, C., Furuya, R. S. & Testi, L. Anatomy of a high-mass star forming cloud: The G24.78+0.08 (proto)stellar cluster. *Astron. Astrophys.* **401**, 227–242 (2003).
21. Beltrán, M. T. *et al.* A detailed study of the rotating toroids in G31.41+0.31 and G24.78+0.08. *Astron. Astrophys.* **435**, 901–925 (2005).
22. Ungerechts, H., Winnewisser, G. & Walmsley, C. M. Ammonia observations and temperatures in the S140/L1204 molecular cloud. *Astron. Astrophys.* **157**, 207–216 (1986).
23. Cesaroni, R., Churchwell, E., Hofner, P., Walmsley, C. M. & Kurtz, S. Hot ammonia towards compact H II regions. *Astron. Astrophys.* **288**, 903–920 (1994).
24. Walmsley, M. Dense cores in molecular clouds. *Rev. Mex. Astron. Astrophys. Conf. Ser.* **1**, 137–148 (1995).
25. Keto, E. On the evolution of ultracompact H II regions. *Astrophys. J.* **580**, 980–986 (2002).
26. Wolfire, M. G. & Cassinelli, J. P. Conditions for the formation of massive stars. *Astrophys. J.* **319**, 850–867 (1987).
27. Bonnell, I. A. & Bate, M. R. Binary systems and stellar mergers in massive star formation. *Mon. Not. R. Astron. Soc.* **362**, 915–920 (2005).
28. McKee, C. F. & Tan, J. C. The formation of massive stars from turbulent cores. *Astrophys. J.* **585**, 850–871 (2003).
29. McKee, C. F. & Tan, J. C. Massive star formation in 100,000 years from turbulent and pressurized molecular clouds. *Nature* **416**, 59–61 (2002).
30. Bonnell, I. A., Vine, S. G. & Bate, M. R. Massive star formation: nurture, not nature. *Mon. Not. R. Astron. Soc.* **349**, 735–741 (2004).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements NRAO is operated by Associated University, Inc., under contract with the National Science Foundation. We thank P. Ho for suggestions that improved the presentation of our results.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to M.T.B. (mbeltran@am.ub.es).

LETTERS

Bose–Einstein condensation of quasi-equilibrium magnons at room temperature under pumping

S. O. Demokritov¹, V. E. Demidov¹, O. Dzyapko¹, G. A. Melkov², A. A. Serga³, B. Hillebrands³ & A. N. Slavin⁴

Bose–Einstein condensation^{1,2} is one of the most fascinating phenomena predicted by quantum mechanics. It involves the formation of a collective quantum state composed of identical particles with integer angular momentum (bosons), if the particle density exceeds a critical value. To achieve Bose–Einstein condensation, one can either decrease the temperature or increase the density of bosons. It has been predicted^{3,4} that a quasi-equilibrium system of bosons could undergo Bose–Einstein condensation even at relatively high temperatures, if the flow rate of energy pumped into the system exceeds a critical value. Here we report the observation of Bose–Einstein condensation in a gas of magnons at room temperature. Magnons are the quanta of magnetic excitations in a magnetically ordered ensemble of magnetic moments. In thermal equilibrium, they can be described by Bose–Einstein statistics with zero chemical potential and a temperature-dependent density. In the experiments presented here, we show that by using a technique of microwave pumping it is possible to excite additional magnons and to create a gas of quasi-equilibrium magnons with a non-zero chemical potential. With increasing pumping intensity, the chemical potential reaches the energy of the lowest magnon state, and a Bose condensate of magnons is formed.

Superfluids and superconductors were, for a long time, the only physical systems where the effect of Bose–Einstein condensation (BEC) had been observed. The recent discovery of BEC in ultracold diluted atomic gases^{5–8} has confirmed that this effect has a general nature. Although BEC of quasi-particles created in a solid by external pumping has been sought for over a decade, an unambiguous confirmation of BEC in such systems is still missing. The most studied system is that of excitons in semiconductors^{9–12}, but work on polaritons¹³ and phonons¹⁴ has also been reported.

A gas of magnons is a very attractive candidate for the BEC transition^{15,16}. It can be considered as consisting of relatively weakly interacting bosons at temperatures far below T_C , the temperature of magnetic ordering. Magnons are reminiscent of Bose particles in a quantum gas of atoms—many magnons can occupy the same quantum state, and the main mechanism of magnon thermalization is four-magnon scattering, which corresponds to particle–particle collision processes. The BEC transition of magnons at low temperatures has been discussed in many publications over the past few years^{17–19}.

Yttrium–iron–garnet (YIG) films comprise a very suitable medium for the experimental investigation of the magnon BEC transition. First, the well-known process of parametric pumping^{20,21} offers an effective way to feed energy into the low-frequency part of the magnon spectrum, which then can relax into magnons with the minimum frequency, ν_m , where BEC should take place. This

frequency, which is mainly determined by the applied magnetic field, is 2–3 GHz for the described experiments ($h\nu_m/k_B \approx 100$ mK; here h is Planck's constant, and k_B is the Boltzmann constant). Using parametric pumping, a pumped magnon density of 10^{18} – 10^{19} cm⁻³ can be reached²¹. Although this density is much smaller than that of thermal magnons at room temperature, 10^{21} – 10^{22} cm⁻³, this increase is enough to cause the BEC transition of magnons, as shown below. Second, YIG films provide a very long spin–lattice relaxation time of above 1 μ s. In contrast, the magnon–magnon thermalization time due to the two- and four-magnon scattering relaxation mechanisms can be as low as 100–200 ns. As both mechanisms keep the number of magnons constant, a quasi-equilibrium state for the magnon gas can be realized with a non-zero chemical potential.

The total magnon density, N_0 , and the total energy density of the magnon system, E_0 , are determined at temperature T_0 by the integrals $N_0(T_0) = \int \rho(\nu) d\nu$ and $E_0(T_0) = \int h\nu \rho(\nu) d\nu$, where $\rho(\nu)$ is the magnon spectral density, which is equal to the product of the magnon density of states, $D(\nu)$, and the statistical occupation function $n(\nu)$:

$$\rho(\nu) = D(\nu)n(\nu) = \frac{D(\nu)}{\exp\left(\frac{h\nu - \mu}{k_B T_0}\right) - 1} \quad (1)$$

Here T_0 is the temperature of the system (room temperature in our case) and μ is the chemical potential. As mentioned above, the chemical potential of a magnon gas in thermodynamic equilibrium with the lattice is zero, as the number of magnons is not conserved, mainly owing to the energy exchange between the magnons and the lattice. The injection of additional magnons by parametric pumping increases both the number of magnons, N , and their total energy, E : $N = N_0 + \delta N$, $E = E_0 + \delta E$. The values δE and δN are directly related to each other: $\delta E = h\nu_p \delta N$, where ν_p is the frequency of the pumped magnons. If magnon–magnon interactions dominate over spin–lattice relaxation, the magnon gas reaches a quasi-equilibrium state with a non-zero chemical potential. Substituting the values typical for the described experiment ($\delta N \approx 5 \times 10^{18}$ cm⁻³ and $\nu_p = 4$ GHz) into the above relations yields $\mu/k_B = 100$ mK. This estimate shows that μ can be made as large as $h\nu_m$, that is, using parametric pumping it is possible to bring the magnon system close to the condition of the BEC transition.

The experimental set-up for magnon excitation in YIG films and their detection using Brillouin light scattering (BLS)²² spectroscopy is shown schematically in Fig. 1. The studies were performed on optically transparent YIG films of 2–10 μ m thickness and lateral sizes of 2 mm $\times$ 20 mm. A microstrip resonator attached to the YIG film creates a microwave pumping field with frequency of $2\nu_p$ in the

¹Institute for Applied Physics, University of Münster, 48149 Münster, Germany. ²Department of Radiophysics, National Taras Shevchenko University of Kiev, 01033 Kiev, Ukraine. ³Fachbereich Physik, Technische Universität Kaiserslautern, 67663 Kaiserslautern, Germany. ⁴Department of Physics, Oakland University, Rochester, Michigan 48309, USA.

range of 6–9 GHz. To avoid thermal overheating of the sample via microwave radiation, pumping was performed in an intermittent pulsed mode with an on/off ratio of 1/20 and a pulse width in the range 1–100 μ s. The film was placed in a uniform static magnetic field, H , up to 1 kOe. The pumping process is illustrated in Fig. 1 inset. The low-frequency part of the spectrum of the magnons with wavevectors parallel to the static magnetization is shown by the solid line in the log-log plot. Those magnons have the lowest frequencies among all the magnons²³, and a characteristic frequency minimum exists in their dispersion law^{24,25}. A microwave photon with a frequency of $2\nu_p$ creates two primary excited magnons of frequency ν_p and opposite wavevectors. These primary magnons relax very fast and create a quasi-equilibrium distribution of thermalized magnons, forming the magnon gas described by equation (1). As the chemical potential of the gas increases with pumping power, a possible BEC transition can take place near the minimum in the spectrum, as it corresponds to the state with the absolute minimum in magnon energy.

To examine the distribution of the magnons over the spectrum, BLS spectroscopy²² was used. As shown in Fig. 1, the incident laser beam is focused onto the resonator. The beam passes through the YIG film, is reflected by the resonator, and passes through the film again. Then the light is collected by a wide-aperture objective lens and sent to the interferometer for frequency analysis of light photons inelastically scattered by the magnons. This approach allows a simultaneous detection of the magnons in a wide interval of in-plane wavevectors, estimated as $\pm 2 \times 10^5 \text{ cm}^{-1}$, which exceeds k_m , as indicated by the red hatching in Fig. 1 inset. Thus, our BLS set-up is able to detect all magnons at and close to the frequency minimum, where the condensation should take place. The BLS experiments are performed with time resolution, wherein the start of the pumping pulse plays the role of the reference stroboscopic clock. The time evolution of $n(\nu)$ after the start of pumping is determined using time frames of 100 ns width.

From the general point of view, the scattering intensity at a given frequency, $I(\nu)$, is directly proportional to the reduced spectral density of scatterers (in our case magnons)²⁶, $I(\nu) \propto \tilde{D}(\nu) = \tilde{D}(\nu)n(\nu)$, where the reduced density of states $\tilde{D}(\nu)$ is calculated by integration over the wavevectors of only the particles accessible in the

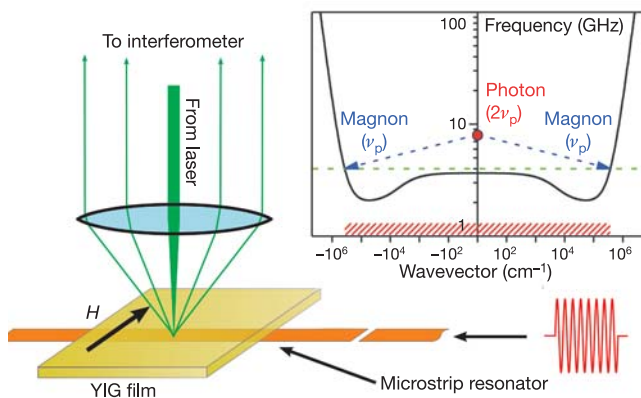


Figure 1 | The set-up for magnon excitation and detection. The resonator attached to the bottom of the yttrium–iron–garnet (YIG) film is fed by microwave pulses. The laser beam is focused onto the resonator, and the scattered light is directed to the interferometer. Inset, the process of creation of two magnons by a microwave photon. The low-frequency part of the magnon spectrum for the applied field H , parallel to the film surface is shown by the solid line. It has a minimum at the wavevector $k_m = 5 \times 10^4 \text{ cm}^{-1}$. The wavevector interval indicated by the red hatching corresponds to the interval of the wavevectors accessible for Brillouin light scattering (BLS).

experiment. Thus, the occupation function of magnons, $n(\nu)$, can be obtained from the BLS experiments, provided their reduced density of states, $\tilde{D}(\nu)$, has been once determined independently.

The experiments were performed over a wide range of experimental conditions. Here we present the results corresponding to $H = 700 \text{ Oe}$ and $\nu_p = 4.05 \text{ GHz}$, which are typical for the range of $H = 600\text{--}800 \text{ Oe}$ and $\nu_p = 3\text{--}4.5 \text{ GHz}$. Figure 2 demonstrates a BLS spectrum of magnons without pumping; such magnons always exist in the sample owing to thermal fluctuation. The spectrum shows a minimum magnon frequency of $\nu_m = 2.1 \text{ GHz}$ ($h\nu_m/k_B = 101 \text{ mK}$) and a maximum cut-off frequency of 3.7 GHz, caused by the finite interval of the magnon wavevectors accessible in the experiment. This spectrum was used to independently determine the reduced density of states function, $\tilde{D}(\nu)$, as the calculated function $\tilde{D}(\nu)$ (ref. 24) contains the value of the surface anisotropy of the YIG film, which is not known with the adequate accuracy. In fact, the BLS spectrum from thermally excited magnons corresponds to the known occupation function, $n(\nu)$, defined by equation (1) with $\mu = 0$. Using the value of the surface anisotropy and the proportionality factor between $I(\nu)$ and $\tilde{D}(\nu) = \tilde{D}(\nu)n(\nu)$ (the vertical scaling factor of the spectra) as the two fitting parameters, the measured spectrum has been fitted. The result of the fit is shown in Fig. 2 by the solid line, whereas the obtained function $\tilde{D}(\nu)$ is shown by the dashed line. We should emphasize that once determined from this fit, $\tilde{D}(\nu)$ has been used for the description of BLS from pumped magnons.

Figure 3 shows the BLS spectra of the pumped magnons recorded at different delay times, τ , as indicated, for pumping pulse duration 1 μ s, and repetition period 20 μ s. From the figure, it is seen that pumping continuously increases the number of magnons in the system with time (note the different vertical scales of the graphs). As four-magnon scattering is a nonlinear process, the thermalization time of the pumped magnons is inversely proportional to the magnon density. Figure 3a shows the data corresponding to $\tau = 200 \text{ ns}$ and two different pumping powers, $P = 4.0 \text{ W}$ (open circles) and 5.9 W (filled circles). The solid lines show the results of the fits based on equation (1) with the chemical potential being the fitting parameter. As seen in Fig. 3a, the data for $P = 4.0 \text{ W}$ cannot be described using the Bose–Einstein statistics, illustrating that the thermalization process at those magnon densities lasts more than 200 ns. By contrast, the data for the pumping power $P = 5.9 \text{ W}$ are described very well by the Bose–Einstein statistics at room temperature and a non-zero chemical potential μ , $\mu/k_B = 98 \pm 1 \text{ mK}$. Thus, for this pumping power the magnon–magnon interaction is fast enough to provide an efficient means for magnon thermalization for

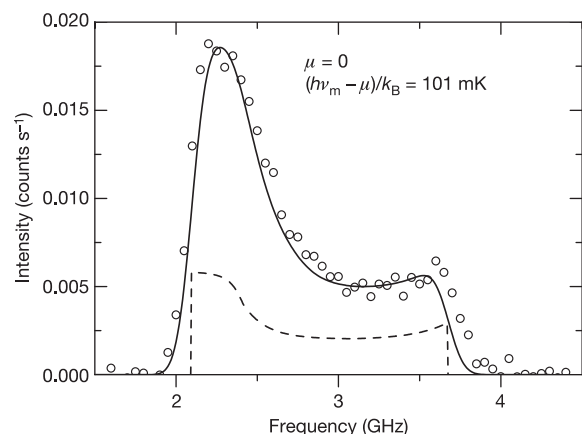


Figure 2 | BLS spectrum of thermal magnons recorded without pumping. The reduced density of states, $\tilde{D}(\nu)$, obtained from the fit of the experimental data (solid line) using equation (1) with the zero chemical potential, μ , is shown by the dashed line. ν_m is the minimum frequency of magnons, h is Planck's constant, and k_B is the Boltzmann constant.

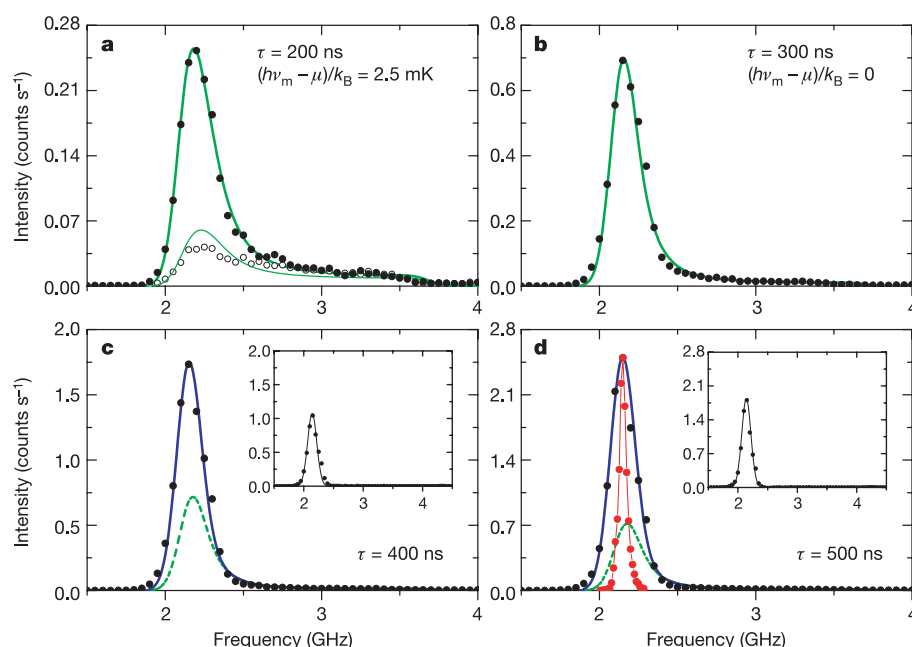


Figure 3 | BLS spectra from pumped magnons at different delay times, τ . **a**, $\tau = 200$ ns; **b**, 300 ns; **c**, 400 ns; and **d**, 500 ns. Black and red filled circles (all panels) show data points recorded at pumping power $P = 5.9$ W, whereas open circles (panel **a**) represent the data recorded at $P = 4$ W. Green solid lines in **a** and **b** show the results of the fit of the spectra based on equation (1) with the chemical potential being a fitting parameter. The fit of

the spectra in **c** and **d** (blue solid lines) are the sums of the magnon density calculated using equation (1) (green dashed line) with $\mu = h\nu_m$ and the magnon density due to the singularity at $\nu = \nu_m$. Red circles in **d** indicate data obtained with a resolution of 50 MHz; red line is a guide for the eye, connecting the red circles. Insets in **c** and **d** illustrate the difference between the corresponding raw spectra and that at $\tau = 300$ ns; axes as main panels.

delay times above 100 ns. All the results discussed below correspond to this pumping power. From comparison of Fig. 2 and different panels of Fig. 3 with each other, we conclude that the magnon occupation is shifted towards lower frequencies as the number of the pumped magnons increases with time. The spectrum shown in Fig. 3b also follows the Bose–Einstein distribution, equation (1) with a non-zero μ , $\mu/k_B = 101 \pm 0.5$ mK (that is, $\mu \approx h\nu_m$), as shown by the solid line. An important intermediate conclusion is that the chemical potential increases with time, reflecting the growth of the magnon density caused by the pumping. For the above experimental conditions, it approaches $h\nu_m$ at $\tau = 300$ ns.

The spectra presented in Fig. 3c and d differ significantly from those shown in Fig. 3a and b. They cannot be fitted using the above procedure based on equation (1). In fact, we obtain the highest possible magnon density at a critical value $\mu = h\nu_m$. The calculated curves are shown in Fig. 3c and d by the green dashed lines. As shown in the insets, the magnon spectral densities at $\tau = 400$ and 500 ns deviate from those at $\tau = 300$ ns just in the vicinity of ν_m , whereas far from ν_m the difference is within the experimental error. The finite width of the profiles in the insets is due to the finite frequency resolution of the interferometer, $\Delta\nu = 250$ MHz. To reach agreement with the experiment, we had to add a singularity peak $\propto \delta(\nu - \nu_m)$ to the occupation function, $n(\nu)$. The corresponding fits are shown by the blue solid lines. The agreement between the experimental data and the results of the calculation is convincing. Thus, to describe the experimental data we need to postulate the existence of a Bose–Einstein condensate.

For the above experimental conditions, the growth of the magnon density saturates after $\tau = 500$ ns. This is connected with the fact that stationary flow balance is achieved between the excitation of the primary magnons by the pumping, their relaxation into other magnons, condensation of those magnons in the condensate and the final energy relaxation to the lattice. After that, the magnon gas stays in the quasi-equilibrium state for the rest of the pumping pulse duration, which can be as long as 100 μ s. Thus the ratio of the lifetime of the prepared magnon system to the thermalization time can be

made as large as 500, which is at least comparable with the corresponding ratio found for the case of alkali atoms⁵.

In order to estimate the upper limit for the width of the observed BEC peak in wavevector space, Δk_{max} , additional experiments with the maximum achievable frequency resolution of the spectrometer have been performed (shown in Fig. 3d in red), confirming that the frequency width of the BEC peak is below 50 MHz. Using the known dispersion law of magnons, we can estimate Δk_{max} as 10^4 cm^{-1} , or 10^{-3} reciprocal lattice units (r.l.u.). By contrast, the thermal magnons are distributed over the Brillouin zone up to the wavevector k_T corresponding to the magnons with thermal energy; that is, $k_T \approx 0.5$ r.l.u. at room temperature. Thus, the observed reduction of width of the magnon distribution in wavevector space due to the condensation effect is more than two orders of magnitude.

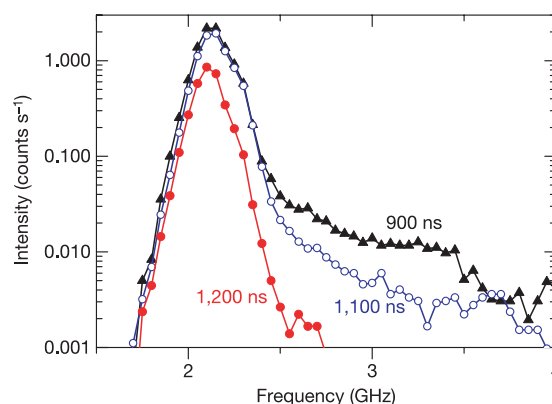


Figure 4 | Evolution of the magnon population after the pumping is switched off at $\tau = 1,000$ ns. Open and filled circles, spectrum of light scattered from pumped magnons at $\tau = 1,100$ ns and 1,200 ns, respectively. Triangles, spectrum corresponding to the stationary pumped state ($\tau = 900$ ns), given for comparison. Note the drastic decay of the density of the non-condensed magnons with time.

The relaxation of the magnon gas after the pumping pulse terminates is illustrated in Fig. 4. The figure explicitly demonstrates that magnons that do not belong to the condensate disappear mostly during the first 100–200 ns after the pulse terminates, whereas the density of the condensate changes slowly. This effect is a consequence of the fact that the condensate is a stable eigenstate of the magnon system, neglecting the weak dissipation to the lattice. By contrast, the non-condensed magnons keep scattering into the condensate owing to the four-magnon scattering mechanism. Finally, at delays above 10 μ s the magnon system comes again into equilibrium with the lattice and is characterized by a spectrum similar to that shown in Fig. 2.

The observed condensation of pumped magnons demonstrates intrinsic features of BEC. However, the observed condensate differs in some aspects from those seen in other systems: first, it is found in quasi-equilibrium, which means that the maximum condensate density is governed by the pumping power; and second, it is observed when the lattice is at room temperature. In this way, our findings contribute both to the quantum thermodynamics and to the thermodynamics of non-equilibrium systems in general. They open a new route for the investigation of thermodynamics in systems of quasi-particles by showing that a non-zero chemical potential can be changed independently of the system temperature.

Received 15 May; accepted 21 July 2006.

1. Bose, S. N. Planck's Gesetz und Lichtquantenhypothese. *Z. Phys.* **26**, 178–181 (1924).
2. Einstein, A. Quantentheorie des einatomigen idealen Gases. Part I. *Sber. Preuss. Akad. Wiss.* **22**, 261–267 (1924); Quantentheorie des einatomigen idealen Gases. Part II. *Sber. Preuss. Akad. Wiss.* **1**, 3–14 (1925).
3. Fröhlich, H. Bose condensation of strongly excited longitudinal electric modes. *Phys. Lett. A* **26**, 402–403 (1968).
4. Mesquita, M. V., Vasconcellos, A. R. & Luzzi, R. Positive-feedback-enhanced Fröhlich's Bose–Einstein-like condensation in biosystems. *Int. J. Quant. Chem.* **66**, 177–187 (1998).
5. Anderson, M. H., Ensher, J. R., Matthews, M. R., Wieman, C. E. & Cornell, E. A. Observation of Bose–Einstein condensation in a dilute atomic vapor. *Science* **269**, 198–201 (1995).
6. Davis, K. B. *et al.* Bose–Einstein condensation in a gas of sodium atoms. *Phys. Rev. Lett.* **75**, 3969–3973 (1995).
7. Bloch, I., Hänsch, T. W. & Esslinger, T. Measurement of the spatial coherence of a trapped Bose gas at the phase transition. *Nature* **403**, 166–170 (2000).
8. Hancox, C. I., Doret, S. C., Hummon, M. T., Luo, L. J. & Doyle, J. M. Magnetic trapping of rare-earth atoms at millikelvin temperatures. *Nature* **431**, 281–284 (2004).
9. Keldysh, L. V. The electron-hole liquid in semiconductors. *Contemp. Phys.* **27**, 395–428 (1986).
10. Butov, L. V. Condensation and pattern formation in cold exciton gases in coupled quantum wells. *J. Phys. Condens. Matter* **16**, R1577–R1613 (2004).
11. Fukuzawa, T., Mendez, E. E. & Hong, J. M. Phase transition of an exciton system in GaAs coupled quantum wells. *Phys. Rev. Lett.* **64**, 3066–3069 (1990).
12. Eisenstein, J. P. & MacDonald, A. M. Bose–Einstein condensation of excitons in bilayer electron systems. *Nature* **432**, 691–694 (2004).
13. Yamamoto, Y. Half-matter, half-light amplifier. *Nature* **405**, 629–630 (2000).
14. Misochko, O. V., Hase, M., Ishioka, K. & Kitajima, M. Transient Bose–Einstein condensation of phonons. *Phys. Lett. A* **321**, 381–387 (2004).
15. Kalafati, Y. D. & Safonov, V. L. Thermodynamic approach to the theory of paramagnetic resonance of magnons. *Zh. Eksp. Teor. Phys.* **95**, 2009–2020 (1989); [in English] *Sov. Phys. JETP* **68**, 1162 (1989).
16. Kaganov, M. I., Pustynnik, N. B. & Shalaeva, T. I. Magnons, magnetic polaritons, magnetostatic waves. *Phys. Usp.* **40**, 181–224 (1997).
17. Nikuni, T., Oshikawa, M., Oosawa, A. & Tanaka, H. Bose–Einstein condensation of dilute magnons in TiCuCl_3 . *Phys. Rev. Lett.* **84**, 5868–5871 (2000).
18. Rüegg, C. *et al.* Bose–Einstein condensation of the triplet states in the magnetic insulator TiCuCl_3 . *Nature* **423**, 62–65 (2003).
19. Della Torre, E., Bennett, L. H. & Watson, R. E. Extension of the Bloch $T^{3/2}$ law to magnetic nanostructures: Bose–Einstein condensation. *Phys. Rev. Lett.* **94**, 147210 (2005).
20. L'vov, V. S. *Wave Turbulence Under Parametric Excitation* (Springer, Berlin, 1994).
21. Gurevich, A. G. & Melkov, G. A. *Magnetization Oscillations and Waves* (CRC, New York, 1996).
22. Demokritov, S. O., Hillebrands, B. & Slavin, A. N. Brillouin light scattering studies of confined spin waves: linear and nonlinear confinement. *Phys. Rep.* **348**, 441–489 (2001).
23. Damon, R. W. & Eshbach, J. R. Magnetostatic modes of a ferromagnet slab. *J. Phys. Chem. Solids* **19**, 308–320 (1961).
24. Sparks, M. Ferromagnetic resonance in thin films. I. Theory of normal-mode frequencies. *Phys. Rev. B* **1**, 3831–3856 (1970).
25. Wolfram, T. & De Wames, R. E. Magnetoexchange branches and spin-wave resonance in conducting and insulating films: perpendicular resonance. *Phys. Rev. B* **4**, 3125–3141 (1971).
26. Cottam, M. & Lockwood, D. *Light Scattering in Magnetic Solids* (Wiley, New York, 1986).

Acknowledgements Support by the Deutsche Forschungsgemeinschaft, the US Army Research Office, and the Science & Technology Center of Ukraine is acknowledged. We are also indebted to D. Mills, V. Safonov and M. Katsnelson for discussions.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to S.O.D. (demokrit@uni-muenster.de).

LETTERS

Violation of the incompressibility of liquid by simple shear flow

Akira Furukawa¹ & Hajime Tanaka¹

In standard fluid dynamics^{1,2}, the density change associated with flow is often assumed to be negligible, implying that the fluid is incompressible. For example, this has been established for simple shear flows, where no pressure change is associated with flow: there is no volume deformation due to viscous stress and inertial effects can be neglected. Accordingly, any flow-induced instabilities (such as cavitation) are unexpected for simple shear flows. Here we demonstrate that the incompressibility condition can be violated even for simple shear flows, by taking into account the coupling between the flow and density fluctuations, which arises owing to the density dependence of the viscosity. We show that a liquid can become mechanically unstable above a critical shear rate that is given by the inverse of the derivative of viscosity with respect to pressure. Our model predicts that, for very viscous liquids, this shear-induced instability should occur at moderate shear rates that are experimentally accessible. Our results explain the unusual shear-induced instability observed in viscous lubricants^{3–5}, and may illuminate other poorly understood phenomena associated with mechanical instability of liquids at low Reynolds number; for example, shear-induced cavitation and bubble growth⁶, and shear-banding of very viscous liquids such as metallic glasses⁷ and the Earth's mantle⁸.

Bubbles often vigorously spout out when we pour or shake a bottle of champagne or cola. When on a boat, we also see that enormous gas bubbles are formed around a rotating screw. These flow-induced cavitation phenomena have been qualitatively explained by Bernoulli's law as follows^{9,10}: when a pressure drop due to high-speed flow makes the pressure in a liquid lower than its vapour pressure, cavitation occurs. This scenario of inertial pressure drop and the resulting cavitation works for flow at rather high Reynolds number, *Re*.

Even for low-*Re* flow, flow-induced instability of liquids—which manifests either as cavitation (as in the above examples) or as shear banding or shear localization—is widely observed. Very viscous liquids such as glassy materials and mantle often exhibit shear banding under flow (flow-induced instability of material)^{7,8}. Liquid lubricants cavitate under high pressures and high shear stresses. Bair *et al.*^{3–5} revealed, by systematic experimental studies combining rheological measurements and direct microscopy observations, that shear flow induces cavitation, accompanied by marked shear-thinning behaviour. In low-molecular-weight unentangled polymer melts, cavity formation under simple shear was also observed⁶. Liquids just before cavitation behave as (linear) newtonian fluids, which indicates that this phenomenon is not due to constitutive (visco-elastic) instability^{11–16}. Thus, a new scenario of liquid instability under simple shear is necessary to account for these phenomena, which are of fundamental and practical importance.

A cavitation criterion for sheared fluids has been proposed by several researchers independently^{4,17}. According to their criterion, cavitation occurs if the maximum principal stress created by shear

deformation, estimated as $\sim(\eta\dot{\gamma} - p)$, becomes positive. Their analyses are based on the standard Navier–Stokes equation of incompressible fluids in a steady state. For such a framework, however, simple shear flow does not accompany any volume change (density fluctuations); in other words, a shear mode never couples with a longitudinal one. Thus, although their explanation is intuitively appealing, we cannot expect instability of liquid by simple shear. In this Letter, we examine how simple shear deformation can be coupled with density fluctuations. We propose a new mechanism of flow-induced instability of liquid matter, or a new criterion for the violation of the incompressibility condition.

The dynamics of compressible fluids can be described by the following hydrodynamic equations^{1,2}. The density $\rho(\mathbf{r}, t)$ obeys the continuity equation:

$$\frac{\partial}{\partial t}\rho = -\nabla \cdot (\rho \mathbf{v}) \quad (1)$$

where $\mathbf{v}$ is the velocity field. Then the velocity field obeys the Navier–Stokes equation:

$$\rho \left(\frac{\partial}{\partial t} + \mathbf{v} \cdot \nabla \right) \mathbf{v} = -\nabla \cdot \vec{\Pi} + \nabla \cdot \vec{\sigma} \quad (2)$$

In equation (2), $\vec{\Pi}(\mathbf{r}, t)$ is the pressure tensor, which is determined from the equation of state under a local equilibrium assumption. The pressure tensor can be expressed up to the linear order of density fluctuation $\delta\rho$ from the average ρ_0 , as:

$$\Pi_{ij} = \left(p_0 + K_T^{-1} \frac{\delta\rho}{\rho_0} \right) \delta_{ij} \quad (3)$$

where p_0 is the average pressure and $K_T = (\partial\rho/\partial p)_T/\rho$ is the isothermal compressibility (for an expression for $\vec{\Pi}$ including nonlinear and nonlocal contributions; see Methods). $\vec{\sigma}(\mathbf{r}, t)$ is the viscous stress tensor expressed as:

$$\sigma_{ij} = \eta(\rho) \left(\nabla_i v_j + \nabla_j v_i - \frac{2}{d} \delta_{ij} \partial_m v_m \right) + \delta_{ij} \zeta(\rho) \partial_m v_m \quad (4)$$

where d is the spatial dimension and $\eta(\rho)$ and $\zeta(\rho)$ are the shear and bulk viscosities, respectively, which in general depend on the density ρ . We emphasize that the constitutive relation in equation (4), which is commonly used for simple fluids, does not involve any viscoelastic (or structural) relaxation. We will show below that it is this density dependence of η that leads to a coupling between density fluctuations and shear flow.

Now we consider a mechanism of shear-induced instability of liquid on the basis of a linear analysis of the above set of standard equations. For shear flow, we set the x axis along the direction of the mean flow and the y axis along the direction of the mean velocity gradient. Then, the mean velocity profile is given by $\langle \mathbf{v}(\mathbf{r}, t) \rangle = \dot{\gamma} y \hat{\mathbf{x}}$,

¹Institute of Industrial Science, University of Tokyo, 4-6-1 Komaba, Tokyo 153-8505, Japan.

where $\hat{x}$ is the unit vector along the x axis. From equation (2), the linearized equation of motion for the volume dilation rate $Z = \nabla \cdot \mathbf{v}$ is derived in the Fourier representation as:

$$\rho_0 \left(\frac{\partial Z_{\mathbf{k}}}{\partial t} - \dot{\gamma} k_x \frac{\partial Z_{\mathbf{k}}}{\partial k_y} + 2i\dot{\gamma} k_x v_{y\mathbf{k}} \right) = - \left(\zeta_0 + \frac{2d-2}{d} \eta_0 \right) k^2 Z_{\mathbf{k}} + \frac{1}{\rho_0 K_T} k^2 \rho_{\mathbf{k}} - 2 \left(\frac{\partial \eta}{\partial \rho} \right) \dot{\gamma} k_x k_y \rho_{\mathbf{k}} \quad (5)$$

where $\eta_0 = \eta(\rho_0)$ and $\zeta_0 = \zeta(\rho_0)$. Here, $Z_{\mathbf{k}}(t) = \int d\mathbf{r} Z(\mathbf{r}, t) e^{-i\mathbf{k}\cdot\mathbf{r}}$ and $\rho_{\mathbf{k}}(t) = \int d\mathbf{r} \rho(\mathbf{r}, t) e^{-i\mathbf{k}\cdot\mathbf{r}}$. In equation (5), there are several terms depending on the shear rate $\dot{\gamma}$. The terms on the left-hand side represent only advection by the mean flow, whereas the last term on the right-hand side causes a coupling between density fluctuations and the mean shear flow. By arranging the last two terms on the right-hand side in equation (5), we here introduce the following effective compressibility K_T^{eff} :

$$\frac{1}{K_T^{\text{eff}}} = \frac{1}{K_T} \left[1 - 2\rho_0 K_T \left(\frac{\partial \eta}{\partial \rho} \right) \dot{\gamma} \hat{k}_x \hat{k}_y \right] \quad (6)$$

where $\hat{k}_x$ and $\hat{k}_y$ are the x and y components of a unit wave vector $\hat{\mathbf{k}}$. From this, we can see that the system softens in the direction of $\hat{k}_x = \hat{k}_y$ (45° from the mean flow direction) under shear flow. Above the critical shear rate $\dot{\gamma}_c$, which is given by:

$$\dot{\gamma}_c = \left[\rho_0 K_T \left(\frac{\partial \eta}{\partial \rho} \right) \right]^{-1} = \left(\frac{\partial \eta}{\partial \rho} \right)_T^{-1} \quad (7)$$

the effective isothermal compressibility becomes ‘negative’; that is, the homogeneous state becomes unstable (shear-induced instability). Our theory indicates that this instability is induced by a coupling

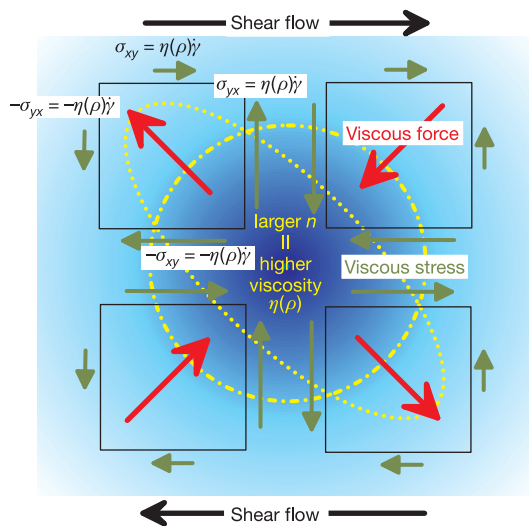


Figure 1 | Intuitive explanation for the mechanism of shear-induced instability of a liquid. For a liquid of non-zero $\partial\eta/\partial\rho$, density fluctuations induce spatial inhomogeneity of viscosity. Here we assume $\partial\eta/\partial\rho > 0$, which is the case for most existing liquids (examples of the exceptions are water and silica). In the figure, the density of the liquid is higher at the central part owing to thermal fluctuations and shear flow is applied as indicated by the black arrows. Viscous stresses acting on the boundaries of squares are shown as green arrows. The sum of these viscous stresses acting on each region are shown by thick red arrows. Note that such forces are absent for $\partial\eta/\partial\rho = 0$, which has been assumed so far. The inhomogeneous viscous force tends to enhance density (pressure) fluctuations. The denser region becomes narrower along the $\hat{k}_x = \hat{k}_y$ direction (inhomogenization) and broader in the orthogonal direction (homogenization). This deformation is opposite to that due to affine convection. This feature tends to form a density fluctuation mode, whose wave vector aligns along the $\hat{k}_x = \hat{k}_y$ direction. For a high shear rate above $\dot{\gamma}_c$, this viscous (destabilizing) force wins over the thermodynamic (stabilizing) force, which makes a liquid unstable.

of shear flow to the longitudinal modes—namely, the density fluctuations—which has been overlooked so far. An intuitive explanation is given in Fig. 1. It may be worth mentioning advection effects in simple shear flow. In a simple shear flow fluid elements experience extension and compression at, respectively, plus and minus 45°. This advection effect may weaken the destabilization mechanism described above. To make this prediction more complete, we need to perform a full linear stability analysis of equation (5), including the advective terms. Instead of doing so, however, we will show a full numerical analysis of equations (1) to (4) later, including all the advective and nonlinear terms.

Here we briefly discuss the implication of our mechanism on the incompressibility condition of fluid, which is one of the key concepts of fluid dynamics^{1,2}. The density change associated with flow with velocity V , $\Delta\rho$, can be estimated from the pressure change Δp . For high-Re flow, Bernoulli's law tells us that the amount of a pressure drop induced by flow is $\Delta p \approx (1/2)\rho V^2$. For this case, $\Delta\rho/\rho_0 \approx K_T \Delta p \approx M^2$. Here we used the following relations: the sound velocity $c_s = 1/\sqrt{\rho_0 K_T}$ and the Mach number $M = V/c_s$. On the other hand, for low-Re flow, which is considered in this Letter, the pressure drop Δp is induced by the viscous stress: $\Delta p \approx \eta V/L$ (where L is the characteristic length of the system). Thus, $\Delta\rho/\rho_0 \approx K_T \eta V/L = M^2/\text{Re}$, where $\text{Re} = \rho_0 L V/\eta$ (see pages 64–69 in ref. 2).

To sum up, the incompressible condition is given by $M^2 \ll 1$ for large Re, and by $M^2 \ll \text{Re}$ for low Re (strictly speaking, we also need the condition $p - \Delta p > p_v$, where p_v is the vapour pressure, which will prevent cavitation)^{9,10}. We note that the latter condition is usually satisfied for low-Re flow. Furthermore, it has been believed that we can always assume incompressibility for steady simple shear on the basis of the following argument. If we neglect the density dependence of the viscosity, equation (2) reduces to the standard Navier–Stokes equation:

$$\rho \left(\frac{\partial}{\partial t} + \mathbf{v} \cdot \nabla \right) \mathbf{v} = -\nabla \cdot \vec{\Pi} + \eta_0 \nabla^2 \mathbf{v} + \left(\zeta_0 + \frac{d-2}{d} \eta_0 \right) \nabla \nabla \cdot \mathbf{v} \quad (8)$$

In this case, because there are neither inertial and viscous pressure changes for simple shear, we do not expect shear enhancement of density fluctuations. In contrast we demonstrated above that the density dependence of viscosity (or, more generally, that of viscous stress) allows us to enhance density fluctuations. Thus, we need an additional condition before we can assume incompressibility: $\dot{\gamma} \ll \dot{\gamma}_c$.

We emphasize that our argument relies solely on the well-established classical hydrodynamic theory. Most existing liquids have non-zero $(\partial\eta/\partial p)_T$. Particularly important are the following two cases, where $\dot{\gamma}_c$ can become a small value: (1) soft liquids with large K_T and (2) viscous liquids, whose $(\partial\eta/\partial p)_T$ is very large. An example of soft liquids is a fluid near the gas–liquid critical point¹², for which the isothermal compressibility diverges as $K_T \approx \Gamma(T/T_c - 1)^{-\gamma}$, where T_c is the critical temperature, $\gamma \approx 1.24$ is the critical exponent, and Γ is a material-dependent constant. For a weak shear, it has been established that shear-induced mixing (shear-induced stabilization of a homogeneous phase) occurs near the criticality^{12,13}. This is because density fluctuations are suppressed by shear, if $\dot{\gamma}$ exceeds the inverse of the lifetime of the fluctuations. Our theory predicts that the completely opposite effect becomes more and more important for higher shear rates and eventually leads to mechanical instability, that is, destabilization of a homogeneous phase, or cavitation. For example, for xenon at the critical density and at $T = T_c + 3 \text{ mK}$, we have $K_T \approx 0.017 \text{ Pa}^{-1}$ (ref. 18) and $\rho_0(\partial\eta/\partial p)_T \approx 5.7 \times 10^{-5} \text{ Pa s}$ (ref. 19). Therefore, we expect that the application of shear flow of $\dot{\gamma} > \dot{\gamma}_c \approx 10^6 \text{ s}^{-1}$ may induce mechanical instability of the liquid phase. Although the shear rate of the instability might be difficult to access experimentally, we note that even for lower shear rates, a system can be in a nonequilibrium ‘metastable’ state, in which we expect interesting behaviour such as anomalous enhancement of density fluctuations, anisotropic transmission of sound waves, and nucleation and growth of gas bubbles.

Here we also mention the possible relevance of our scenario to the problem of sheared confined liquids, which affects many technological applications in micro- and nanofluidics. Recently the breakdown of the no-slip boundary condition has attracted considerable attention in the field of microfluidics (for reviews, see refs 20, 21). Several researchers attributed it to the formation of nano-bubbles or gas layers at the solid–liquid boundary²² under shear. However, the mechanism of the formation of such gas regions under shear still remains elusive. Because liquids saturated with dissolved gas are unstable even for small density (or pressure) fluctuations, we speculate that even simple shear flow can induce its instability via our mechanism, which further leads to gas-layer formation on a hydrophobic wall surface under the influence of wetting effects.

Next, we consider case (2) for viscous liquids, which is much more important practically than case (1). There are many fluid systems that exhibit severe pressure- (or density-) dependencies of viscosities, including glass-forming liquids: molecular liquids²³, mineral melts²⁴, and metallic glass formers⁷. In Table 1 we compare our prediction with the experimental data on the shear-induced cavitation for liquid lubricants, phenyl ether oligomer (molecular weight $M_w = 450$), by Bair *et al.*^{3–5}. $\dot{\gamma}_c^{\text{ex}}$ is the critical shear rate, above which the linear newtonian behaviour breaks down. The values of both $\dot{\gamma}_c^{\text{ex}}$ and $\dot{\gamma}_c^{\text{th}} = (\partial\eta/\partial p)_T^{-1}$ are estimated from the data in refs 3–5 and 25. Our prediction is consistent with the experimental results. We note that in all conditions the structural relaxation time τ_α estimated from the data using the relation $\tau_\alpha \approx \eta/G$ (where G is the shear elastic modulus) is sufficiently shorter than $1/\dot{\gamma}_c^{\text{ex}}$. Thus, this occurrence of the instability for $\dot{\gamma}\tau_\alpha < 1$ cannot be explained by conventional knowledge of rheology, such as by invoking viscoelastic instability^{11–13}.

In the following, we check the validity of our prediction by numerical simulation of shear-induced instability of a liquid (see Methods). The aim of this simulation is twofold. First, we wish to demonstrate that instability can really be induced by simple shear flow. As mentioned before, if we neglect the ρ -dependence of η as usual, there should not be any couplings between shear and longitudinal modes. Second, we wish to clarify the roles of nonlinear couplings, which we ignore in the above analysis. As mentioned before, the advective terms and the viscous damping term in equation (5) may suppress the density fluctuations and thus shift the predicted critical shear rate $\dot{\gamma}$ to a higher value. This effect will be checked below.

Figure 2 shows the time evolution of the normalized density $\psi(\mathbf{r}, t) = \rho(\mathbf{r}, t)v_0/m$ at $\dot{\gamma} = 0.125$ (see Methods for the definition of m and v_0). This $\dot{\gamma}$ is above $\dot{\gamma}_c (=0.014)$ estimated by the linear analysis (see Methods). We find that a liquid in the stable one-phase region indeed becomes mechanically unstable even under simple shear flow: the liquid is in a thermodynamically stable state, but is mechanically destabilized by simple shear flow. After some transient of the initial process of instability, $t \gtrsim 70$, the large-amplitude density fluctuations are steadily maintained. In Fig. 3a we plot the time evolution of the average variance $\sqrt{\langle\delta\psi^2\rangle}(t)$, the average shear

stress $\langle\Sigma_{xy}\rangle(t)$, and the average first normal stress difference $\langle N_1 \rangle(t)$ at $\dot{\gamma} = 0.125$, where $\Sigma_{ij} = \sigma_{ij} - \Pi_{ij}$ and $N_1 = \Sigma_{xx} - \Sigma_{yy}$. The spatial averages are taken over all the lattice points. It is found that $\langle\Sigma_{xy}\rangle(t)$ exhibits shear-thinning behaviour after the onset of instability. Furthermore, $\langle N_1 \rangle(t)$ almost always has a positive value in the course of the time evolution. This is because the velocity gradient is concentrated on the lower-density regions, which have a lower viscosity (viscosity is density-dependent), while the stress is supported mainly by the higher-density regions; this is asymmetric stress division.

Figure 3b shows $\sqrt{\langle\delta\psi^2\rangle}$, $\langle\Sigma_{xy}\rangle$, the average viscosity $\langle\eta\rangle (= \langle\Sigma_{xy}\rangle/\dot{\gamma})$, and $\langle N_1 \rangle$ as functions of the shear rate $\dot{\gamma}$, where the time averages as well as the spatial averages are taken for the dynamical steady states. For low enough shear rates, $\dot{\gamma} \lesssim \dot{\gamma}_c$, the system exhibits simple newtonian behaviour, $\Sigma_{xy} = \eta_0\dot{\gamma}$, where η_0 is 1.86 (see Methods). For $\dot{\gamma} \gtrsim \dot{\gamma}_c$, on the other hand, shear-thinning behaviour occurs, owing to mechanical instability. Intriguingly, even a simple liquid without any internal degrees of freedom exhibits such highly nonlinear behaviour, including the appearance of the first normal stress difference above $\dot{\gamma}_c$. The critical shear rate of the onset of the nonlinear behaviour is consistent with the theoretically estimated value of $\dot{\gamma}_c$. Thus, we conclude that a thermodynamically stable liquid indeed becomes mechanically unstable even for simple shear flow around $\dot{\gamma} = \dot{\gamma}_c$. The slight deviation (a factor of 1.5) may be due to advection and viscous damping effects, as discussed before. We note that, for a sufficiently high shear rate, the inhomogeneous state is maintained even in a thermodynamically stable one-phase region, by nonlinear interactions among the fluctuations even without the thermal fluctuations.

Finally, it may be worth pointing out the similarity between the shear-induced instability in one-component liquids and the shear-induced phase separation in complex fluids. In the latter phenomenon, the critical shear rate is given by $[(\phi/K_{\text{os}})(\partial\eta/\partial\phi)]^{-1}$ (refs 12–16), which can be rewritten as $(\partial\eta/\partial\Pi_{\text{os}})_T^{-1}$ in the same style as equation (7). Here ϕ is the polymer volume fraction, $\eta(\phi)$ is the ϕ -dependent polymeric viscosity, Π_{os} is the osmotic pressure, and $K_{\text{os}} = \phi(\partial\Pi_{\text{os}}/\partial\phi)_T$ is the bulk osmotic modulus. Applying shear

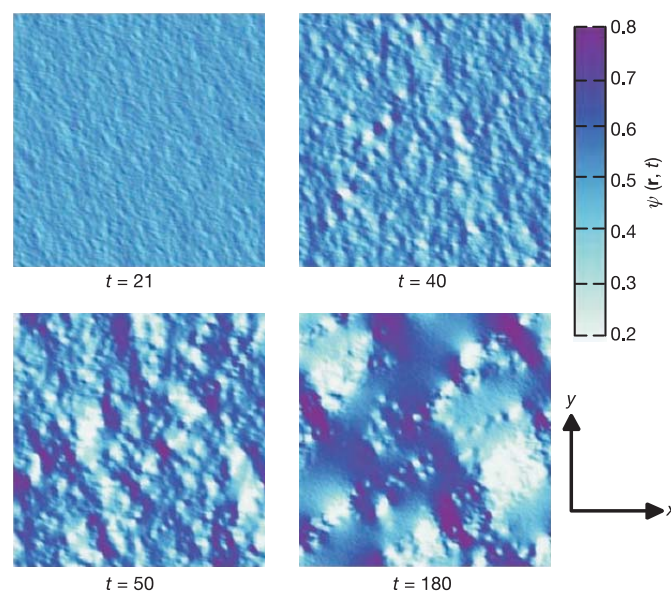


Figure 2 | Shear-induced instability of a thermodynamically stable liquid. The density of a liquid is $\rho_0 = 1.5\rho_c$ (off-critical) and the temperature is slightly above the coexistence curve ($T = 0.945T_c$). We plot the normalized density $\psi(\mathbf{r}, t)$ for various times at $\dot{\gamma} = 0.125$. The bright and dark regions indicate low- and high-density regions, respectively. Initially, the density fluctuations develop along the direction of $k_x = k_y$. Then its amplitude grows. In the late stage, advection and nonlinear effects play a crucial role.

Table 1 | Measured and predicted critical shear rate

T (°C)	ρ (GPa)	$\dot{\gamma}_c^{\text{ex}}$ (s ^{−1})	$\dot{\gamma}_c^{\text{th}}$ (s ^{−1})
5	0.0207	$\sim 2 \times 10^3$	$\sim 5 \times 10^3$
5	0.0310	$\sim 6 \times 10^2$	$\sim 2 \times 10^3$
5	0.0414	$\sim 2 \times 10^2$	$\sim 5 \times 10^2$
20	0.0970	$\sim 2 \times 10^3$	$\sim 4 \times 10^3$
20	0.110	$\sim 5 \times 10^2$	$\sim 10^3$
40	0.260	~ 5	~ 5
60	0.390	~ 3	~ 1

Comparison between the critical shear rate estimated from experimental rheological data measured by a Couette flow device, $\dot{\gamma}_c^{\text{ex}}$, and our theoretical prediction, $\dot{\gamma}_c^{\text{th}} = (\partial\eta/\partial p)_T^{-1}$, for lubricants, phenyl ether oligomer ($M_w = 450$) (refs 3–5). We estimated $\dot{\gamma}_c^{\text{th}}$ directly from the experimental data of the pressure-dependent viscosity reported in refs 5 and 25. The agreement is remarkable despite the large range of $\dot{\gamma}$.

flow of $\dot{\gamma} \approx (\partial\eta/\partial\Pi_{os})^{-1}$, the concentration fluctuations are drastically enhanced. Figure 1 can also be used for this case just by replacing density fluctuations by polymer concentration fluctuations. In a polymer solution, the dynamic asymmetry between the polymers and the solvent molecules is essential for nonequilibrium phenomena, such as shear-induced phase separation^{13–15} and viscoelastic phase separation²⁶. These effects are considered to be characteristic of complex fluids possessing internal elastic degrees of freedom, which can directly be coupled to flow. However, we show here that density fluctuations can be induced by a simple shear flow even in one-component simple liquids, which do not have such internal degrees of freedom. Dynamic asymmetry, or the dependence of η on the density (satisfying the continuity equation), controls the strength of the coupling of flow to density fluctuations. These two examples of simple and complex fluids suggest the universality of our physical scenario for shear-induced instability of liquid matter. There is a common physics between the two with the following correspondence: $\rho \leftrightarrow \phi$ and $p \leftrightarrow \Pi_{os}$. A common principle is that density

fluctuations evolve by a shear-induced mechanical force via $(\partial\eta/\partial\rho)_T$ or $(\partial\eta/\partial\phi)_T$. Finally, the above argument on viscosity variations with density or pressure may further be generalized as the shear stress variations with density or pressure. Then this scenario might be valid even for granular²⁷ and other complex materials.

METHODS

Here we explain the details of our simulation. We use a simple phenomenological fluid model to retain the generality. It can in principle be applied to the whole range of a fluid state including points far from criticality. We express the gas–liquid phase transition by employing the van der Waals theory. The bulk free-energy density is then given by $f(\rho) = -\varepsilon v_0(\rho/m)^2 - (\rho/m)k_B T \ln(m/\rho v_0 - 1)$ (ref. 12), where m is the molecular mass and k_B is the Boltzmann constant. The parameters v_0 and ε are the excluded volume and the magnitude of the attractive potential, respectively. The critical point is given by $\rho_c v_0 = m/3$, $k_B T_c/\varepsilon = 8/27$, and $p_c = \varepsilon/(27v_0)$. Here we neglect temperature inhomogeneity, which should be important under heat flow¹², to extract solely the effects of shear flow on the instability of liquid. Then, the Ginzburg–Landau-type free-energy functional, including the gradient free energy arising from the energy cost associated with the spatial density variation, is:

$$\mathcal{F}\{\rho\} = \int d\mathbf{r} \left[f(\rho) + \frac{1}{2} C |\nabla\rho|^2 \right] \quad (9)$$

where C is a positive constant. Then, the pressure tensor Π_{ij} can be expressed as¹²:

$$\Pi_{ij} = \left(\rho \frac{\partial f}{\partial \rho} - f - \frac{C}{2} |\nabla\rho|^2 - C \rho \nabla^2 \rho \right) \delta_{ij} + C \partial_i \rho \partial_j \rho \quad (10)$$

To take the thermal fluctuations into account, we need to add the divergence of the random stress, $\nabla \cdot \vec{\sigma}^R$, to the right-hand side of equation (2), where the random stress satisfies the fluctuation–dissipation relation¹:

$$\begin{aligned} \langle \sigma_{ij}^R(\mathbf{r}, t) \sigma_{mn}^R(\mathbf{r}', t') \rangle \\ = 2k_B T \left\{ \eta(\rho) (\delta_{im} \delta_{jn} + \delta_{in} \delta_{jm}) + \left[\zeta(\rho) - \frac{2}{d} \eta(\rho) \right] \delta_{ij} \delta_{mn} \right\} \\ \times \delta^d(\mathbf{r} - \mathbf{r}') \delta(t - t') \end{aligned} \quad (11)$$

We consider the conditions $\rho_0 v_0/m = 0.5$ ($\rho_0 = 1.5\rho_c$) and $k_B T/\varepsilon = 0.28$ ($T = 0.945T_c$), which correspond to a thermodynamically stable one-phase state at an off-critical point in the absence of shear flow. The density dependence of the shear viscosity near the critical temperature is assumed to be given by the following expansion up to second order: $\eta(\rho) = \eta_d [1 + \eta_1^* \rho + \eta_2^* \rho^2]$, with η_d being the dilute limit viscosity²⁸. Here we chose $\eta_1^* = 6v_0/m$, $\eta_2^* = 36(v_0/m)^2$, and $\zeta(\rho) = 0.2\eta(\rho)$ (note that $\zeta(\rho)$ does not play an important role in our simulation). Length and time are measured in units of $\ell = (C/v_0 k_B T)^{1/2}$ and $\tau = \eta_c v_0/5k_B T$, respectively, where $\eta_c = \eta(\rho_c) = m/3v_0 = 7\eta_d$ is the viscosity at the critical density. The velocity and stress tensors are scaled by ℓ/τ and η_d/τ , respectively. Furthermore, in our simulation we set $v_0^{1/3}/\ell = 0.1$ and $\eta_d v_0/\ell(k_B T m)^{1/2} = 1$. To avoid cumbersome expressions, we will use the same characters for the scaled variables in the text. In the present system, the critical shear rate is estimated from the linear analysis to be $\dot{\gamma}_c = (\partial\eta/\partial\rho)^{-1} \approx 0.014$. Time integration of equations (1) and (2) is performed on 200×200 square lattices. We impose the periodic boundary condition, and the uniform shear flow $\langle \mathbf{v}(\mathbf{r}, t) \rangle = \dot{\gamma} \mathbf{y} \hat{\mathbf{x}}$ is applied after $t = 0$, using the remesh procedure²⁹.

Received 29 May; accepted 25 July 2006.

- Landau, L. D. & Lifshitz, E. M. *Fluid Mechanics* (Pergamon, New York, 1959).
- Tritton, D. J. *Physical Fluid Dynamics* 2nd edn (Oxford Univ. Press, Oxford, 1988).
- Bair, S. & Winer, W. O. The high shear stress rheology of liquid lubricants at pressures of 2 to 200 MPa. *J. Tribol.* **112**, 246–253 (1990).
- Bair, S. & Winer, W. O. The high pressure, high shear stress rheology of liquid lubricants. *J. Tribol.* **114**, 1–13 (1992).
- Bair, S., Qureshi, F. & Winer, W. O. Observations of shear localization in liquid lubricants under pressure. *J. Tribol.* **115**, 507–514 (1993).
- Archer, L. A., Ternet, D. & Larson, R. G. “Fracture” phenomena in shearing flow of viscous liquids. *Rheol. Acta* **36**, 579–584 (1997).
- Spaepen, F. A microscopic mechanism for steady state inhomogeneous flow in metallic glasses. *Acta Metall.* **25**, 407–415 (1977).
- Alsop, G. I., Holdsworth, R. E., McCaffrey, K. J. W. & Hand, M. (eds) *Flow Processes in Faults and Shear Zones* (Geological Society, London, 2004).
- Brennen, C. E. *Cavitation and Bubble Dynamics* (Oxford Univ. Press, New York, 1995).
- Lohse, D. Bubble puzzles. *Phys. Today* **56**, 36–41 (2003).
- Larson, R. G. *The Structure and Rheology of Complex Fluids* (Oxford Univ. Press, Oxford, 1999).

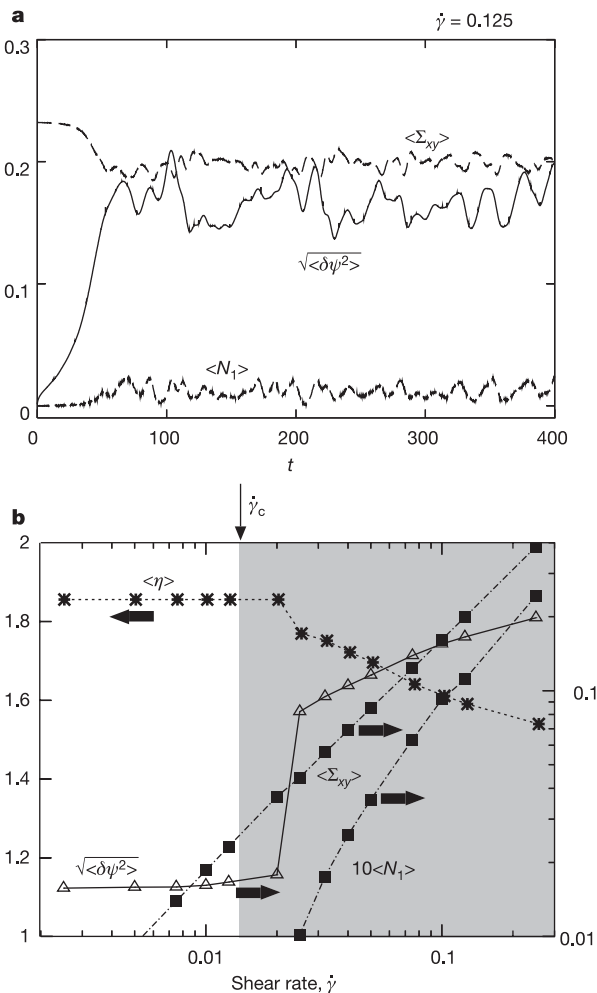


Figure 3 | Rheological behaviour of a thermodynamically stable liquid under shear deformation. **a**, Temporal evolution of the average variance $\sqrt{\langle \delta\psi^2 \rangle}(t)$ (solid curve), the average shear stress $\langle \Sigma_{xy} \rangle(t)$, and the average first normal stress difference $\langle N_1 \rangle$ (dotted curves) after switching on a shear flow of $\dot{\gamma} = 0.125$. It is evident that the inhomogeneous states are steadily maintained with marked shear-thinning behaviour. **b**, $\dot{\gamma}$ -dependence of steady-state values of $\sqrt{\langle \delta\psi^2 \rangle}$, $\langle \Sigma_{xy} \rangle$, $\langle \eta \rangle$ and $\langle N_1 \rangle$. The unstable region predicted by our linear analysis ($\dot{\gamma} \geq \dot{\gamma}_c$) is shaded. For $\dot{\gamma} \geq \dot{\gamma}_c$, strong nonlinear behaviour is clearly seen even for a simple liquid which does not have any internal degrees of freedom. We note that these results reproduce well the basic rheological features experimentally observed for a liquid lubricant, phenyl ether oligomer ($M_w = 450$)³⁰.

12. Onuki, A. *Phase Transition Dynamics* (Cambridge Univ. Press, Cambridge, 2002).
13. Onuki, A. Phase transitions of fluids in shear flow. *J. Phys. Condens. Matter* **9**, 6119–6157 (1997).
14. Helfand, E. & Fredrickson, G. H. Large fluctuations in polymer solutions under shear. *Phys. Rev. Lett.* **62**, 2468–2471 (1989).
15. Milner, S. T. Dynamic theory of concentration fluctuations in polymer solutions under shear. *Phys. Rev. E* **48**, 3674–3691 (1993).
16. Bruinsma, R. & Rabin, Y. Shear-flow enhancement and suppression of fluctuations in smectic liquid crystals. *Phys. Rev. A* **45**, 994–1008 (1992).
17. Joseph, D. D. Cavitation and the state of stress in a flowing liquid. *J. Fluid. Mech.* **366**, 367–378 (1998).
18. Estler, W. T., Hocken, R., Charlton, T. & Wilcox, L. R. Coexistence curve, compressibility, and the equation of state of xenon near the critical point. *Phys. Rev. A* **12**, 2118–2136 (1975).
19. Strumpf, H. J., Collings, A. F. & Pings, C. J. Viscosity of xenon and ethane in the critical region. *J. Chem. Phys.* **60**, 3109–3123 (1974).
20. Granick, S., Zhu, Y. & Lee, H. Slippery questions about complex fluids flowing past solids. *Nature Mater.* **2**, 221–227 (2003).
21. Neto, C., Evans, D. R., Bonaccorso, E., Butt, H.-J. & Craig, V. S. J. Boundary slip in Newtonian liquids: a review of experimental studies. *Rep. Prog. Phys.* **68**, 2859–2897 (2005).
22. de Gennes, P.-G. On fluid/wall slippage. *Langmuir* **18**, 3413–3414 (2002).
23. Roland, C. M., Hensel-Bielowka, S., Paluch, M. & Casalini, R. Supercooled dynamics of glass-forming liquids and polymers under hydrostatic pressure. *Rep. Prog. Phys.* **68**, 1405–1478 (2005).
24. Bottinga, Y. & Richet, P. Silicate melts: The “anomalous” pressure dependence of the viscosity. *Geochim. Cosmochim. Acta* **59**, 2725–2731 (1995).
25. Yasutomi, S., Bair, S. & Winer, W. O. An application of a free volume model to lubricant rheology I—dependence of viscosity on temperature and pressure. *J. Tribol.* **106**, 291–302 (1984).
26. Tanaka, H. Viscoelastic phase separation. *J. Phys. Condens. Matter* **12**, R207–R264 (2000).
27. Hanes, D. M. & Inman, D. L. Observation of rapidly flowing granular-fluid materials. *J. Fluid. Mech.* **150**, 357–380 (1985).
28. Kestin, K., Korfali, O. & Sengers, J. V. Density expansion of the viscosity of carbon dioxide near the critical temperature. *Physica A* **100**, 335–348 (1980).
29. Rogallo, R. S. Numerical experiments in homogeneous turbulence. *NASA Tech. Memo* **81315** (1981).
30. Bair, S. Normal stress difference in liquid lubricants sheared under high pressure. *Rheol. Acta* **35**, 13–23 (1996).

Acknowledgements We are grateful to C. P. Royall for a critical reading of our manuscript. This work was partially supported by a grant-in-aid for JSPS Fellows (A.F.) and a grant-in-aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan (H.T.).

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to H.T. (tanaka@iis.u-tokyo.ac.jp) or A.F. (furu@iis.u-tokyo.ac.jp).

Contribution of anthropogenic and natural sources to atmospheric methane variability

P. Bousquet^{1,2}, P. Ciais¹, J. B. Miller^{3,4}, E. J. Dlugokencky³, D. A. Hauglustaine¹, C. Prigent⁵, G. R. Van der Werf⁶, P. Peylin⁷, E.-G. Brunke⁸, C. Carouge¹, R. L. Langenfelds⁹, J. Lathière¹, F. Papa^{5,10}, M. Ramonet¹, M. Schmidt¹, L. P. Steele⁹, S. C. Tyler¹¹ & J. White¹²

Methane is an important greenhouse gas, and its atmospheric concentration has nearly tripled since pre-industrial times¹. The growth rate of atmospheric methane is determined by the balance between surface emissions and photochemical destruction by the hydroxyl radical, the major atmospheric oxidant. Remarkably, this growth rate has decreased² markedly since the early 1990s, and the level of methane has remained relatively constant since 1999, leading to a downward revision of its projected influence on global temperatures. Large fluctuations in the growth rate of atmospheric methane are also observed from one year to the next², but their causes remain uncertain^{2–13}. Here we quantify the processes that controlled variations in methane emissions between 1984 and 2003 using an inversion model of atmospheric transport and chemistry. Our results indicate that wetland emissions dominated the inter-annual variability of methane sources, whereas fire emissions played a smaller role, except during the 1997–1998 El Niño event. These top-down estimates of changes in wetland and fire emissions are in good agreement with independent estimates based on remote sensing information and biogeochemical models. On longer timescales, our results show that the decrease in atmospheric methane growth during the 1990s was caused by a decline in anthropogenic emissions. Since 1999, however, they indicate that anthropogenic emissions of methane have risen again. The effect of this increase on the growth rate of atmospheric methane has been masked by a coincident decrease in wetland emissions, but atmospheric methane levels may increase in the near future if wetland emissions return to their mean 1990s levels.

The global growth rate of atmospheric methane (CH₄) decreased from nearly $+12 \pm 2$ p.p.b. yr⁻¹ in the 1980s to $+4 \pm 4$ p.p.b. yr⁻¹ in the last decade (all values are means $\pm$ s.d.), but with large year-to-year variations² (Fig. 1a). A peak in growth rate occurred in 1991 in the tropics, followed by a large and abrupt drop in 1992, which began in the northern regions. The past few years have been marked by two positive growth-rate anomalies in 1997–1998 and in 2002–2003, which seem more pronounced north of 30°N than in the tropics.

To understand better why the growth rate of CH₄ has remained persistently smaller after the early 1990s, we have analysed the regional trends in CH₄ differences between sampling sites in the National Oceanic and Atmospheric Association (NOAA) global co-operative air sampling network² and the South Pole site, taken as a reference (Fig. 1b and Supplementary Information). This analysis

suggests that either northern CH₄ emissions have declined persistently since 1992 or that the destruction of CH₄ by the hydroxyl radical (OH) has increased north of 30°N. Several conflicting hypotheses have been proposed to explain interannual and long-term variations in atmospheric CH₄, focusing on wetland CH₄ emissions^{10,13,14}, anthropogenic CH₄ emissions⁵, wild fires^{6–9,15}, OH photochemistry^{3,4,11} and inter-annual wind changes¹². Various models have been used, but the contribution of each process has not been disentangled in a coherent framework, except for short periods^{6,16}. Our understanding of the current methane budget therefore remains plagued by very large uncertainties.

Atmospheric CH₄ measurements can be linked quantitatively to regional sources and sinks by inverse modelling. For the period 1984–2003, the CH₄ concentration responses to the action of OH sinks and regional surface sources were simulated each month with the three-dimensional chemistry transport model LMDZ-INCA¹⁷. The model was forced with interannual analysed winds¹⁸ and inter-annually varying OH concentrations¹⁹. Emissions of CH₄ from different regions of the globe and from distinct processes (see Methods), together with the photochemical sinks, were inferred, and their uncertainties reduced, by matching atmospheric observations within their uncertainties in a Bayesian formalism¹⁹. Clearly, uncertainties in the variations of OH concentrations limit our ability to infer accurately fluctuations in regional CH₄ emissions. The removal of CH₄ by OH nearly balances the sum of all surface sources, making the atmospheric CH₄ budget highly sensitive to OH changes. Thus, we constrained first the interannual variability of OH through a preliminary inversion of methyl chloroform atmospheric observations¹⁹. Contributions of monthly surface CH₄ sources and pre-optimized monthly OH sinks were then combined to fit optimally monthly averages of CH₄ measurements from a global network of 68 sampling sites. Long-term measurements of the ¹³C/¹²C ratio in CH₄ ($\delta^{13}\text{C-CH}_4$) were also used as an additional constraint for the partitioning of microbial-, biomass-burning- and fossil-fuel-related CH₄ sources. We performed a control inversion, supplemented by an ensemble of 17 sensitivity inversions (see Methods).

We found that the year-to-year CH₄ regional flux changes (or anomalies) can be more robustly inverted than their mean values, a result similar to CO₂ inversions^{20,21}. Indeed, among the different sensitivity inversions, the spread of regional flux anomalies is more than a factor of two smaller than the spread of long-term mean fluxes. In other words, possible biases in the inversions seem to have low interannual variability. To illustrate this point, we tested the impact

¹Laboratoire des Sciences du Climat et de l'Environnement, IPSL-LSCE, CEA-CNRS-UVSQ, F-91191, France. ²Université de Versailles Saint Quentin en Yvelines, F-78035, France.

³NOAA Earth System Research Laboratory, Global Monitoring Division, Boulder, Colorado 80305-3328, USA. ⁴Cooperative Institute for Research in Environmental Science, Campus Box 216, University of Colorado, Boulder, Colorado 80309, USA. ⁵LERMA, Observatoire de Paris, F-75014, France. ⁶Faculty of Earth and Life Sciences, Vrije Universiteit, Amsterdam, The Netherlands. ⁷Laboratoire de Biogéochimie Isotopique, LBI, F-78026, France. ⁸South African Weather Service, Stellenbosch 7599, South Africa. ⁹CSIRO, Marine and Atmospheric Research, Victoria 3195, Australia. ¹⁰NASA-GISS-Columbia University, New York, New York 10025, USA. ¹¹Earth System Science Department, University of California, Irvine, California 92697, USA. ¹²Institute of Arctic and Alpine Research, University of Colorado, Boulder, Colorado 80309, USA.

of adding an additional methane source from plants²² with an *a priori* value of $+150 \pm 60 \times 10^{12}$ grams of CH_4 per year ($\text{Tg of CH}_4 \text{ yr}^{-1}$). The long-term-mean inverted fluxes were strongly affected by this 'intrusion' of this new source, but the atmospheric measurements remained fitted and the global budget was conserved, after a reduction in plant and other source emissions (-30%) within their uncertainties. The sparseness of the tropical network, along with large uncertainties on prior emissions, prevents us from verifying the existence of long-term CH_4 emissions by plants. Adding plants in the *a priori* CH_4 sources mix, however, did not alter the inferred anomalies in the 1990s (see Supplementary Information). Given their robustness, the flux anomalies are therefore the primary focus of the following analysis.

We found that fluctuations in wetland emissions are the dominant contribution to interannual variability in surface emissions ($\pm 12 \text{ Tg of CH}_4 \text{ yr}^{-1}$), explaining 70% of the global emission anomalies over the past two decades, as compared with only 15% contributed by biomass burning (Fig. 2). This result disagrees with previous studies suggesting a dominant role of fires^{6–9,14}. As an independent check on the inverted wetland variability, we applied a simple wetland flux model (see Supplementary Information) based on ref. 23 and driven by interannually varying climate data¹⁸ and by estimates of remotely sensed changes in flooded areas for the period 1993–2001. There is good agreement in the magnitude of the wetland flux anomalies between the bottom-up and inversion results (normalized standard

deviation (NSD) = 1.1; see Methods), as seen in Fig. 2. The correlation between the two estimates is improved when a 3-month lag is applied to the inversion results ($r^2 = 0.4$, $P = 0.06$). In 1993–2001, wetland emissions in the bottom-up model show a persistent negative trend of $2.5 \text{ Tg of CH}_4 \text{ yr}^{-1}$, in response to a marked decrease in flooded area worldwide (at a rate of $-1.1\% \text{ yr}^{-1}$ for a mean area of $4.2 \times 10^6 \text{ km}^2$), mostly in temperate and tropical Asia and in tropical South America²³. The inversion infers decreasing wetland emissions after 1993, but with a smaller trend ($-0.6 \text{ Tg of CH}_4 \text{ yr}^{-1}$). Shrinking wetland areas may reflect recurrent dryness observed in the tropics after 1990 (ref. 18), and northward after 1999 (ref. 24).

The inversion attributes global variations in the biomass-burning emissions of the order of $\pm 3.5 \text{ Tg of CH}_4 \text{ yr}^{-1}$ (Fig. 2). In 1989–2002, these anomalies are in very good agreement with independent estimates⁹ derived from remote sensing data after 1996 ($r^2 = 0.6$, $P = 0.012$; NSD = 0.8) and inferred from global CO variations before 1996 ($r^2 = 0.4$, $P = 0.08$; NSD = 1.2; see Supplementary Information). Such agreement is remarkable, given that the *a priori* biomass-burning fluxes prescribed to the inversion are constant from year to year. The members of the inversion ensemble that include $\delta^{13}\text{C-CH}_4$ observations agree best with the magnitude of the bottom-up anomalies⁹ (NSD = 1.1). At face value, these 'isotopic' inversions

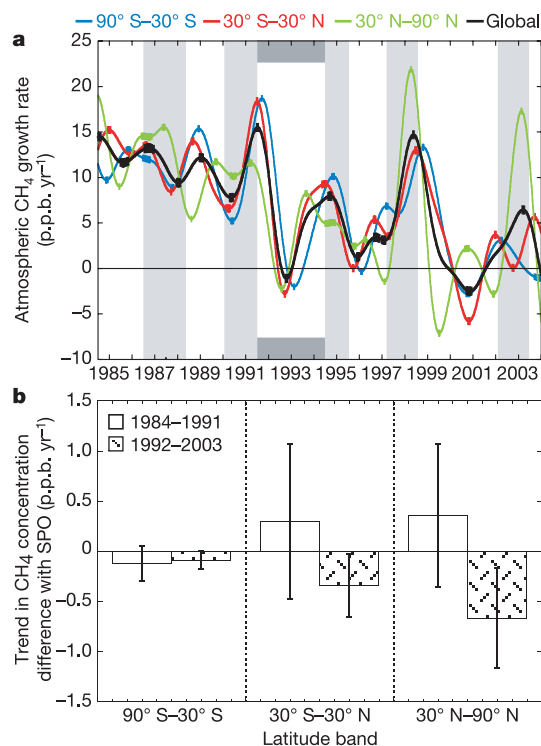


Figure 1 | Variability and trends in atmospheric CH_4 over the past two decades. **a**, Interannual variations in the growth rate of atmospheric CH_4 (p.p.b. yr^{-1}) in the period 1984–2003, calculated by using data from the NOAA air sampling sites used in the inversion model (maximum of 50 sites). Black, global growth rate; blue, Southern Hemisphere $<30^\circ\text{S}$; red, 30°S to 30°N (tropics); green, Northern Hemisphere $>30^\circ\text{N}$; light grey, El Niño episodes; dark grey, anomaly following the Pinatubo climate anomaly. **b**, Average trends in the CH_4 difference (in p.p.b. yr^{-1}) between sites grouped in three latitude bands and the South Pole site (SPO). Trends were calculated by using monthly deseasonalized observations. Open bars indicate trends in ΔCH_4 in the 1980s (1984–1991), when the global growth rate was $+12 \pm 2$ p.p.b. yr^{-1} ; hatched bars indicate trends in ΔCH_4 after 1993, when the global growth rate was 4 ± 4 p.p.b. yr^{-1} . Error bars are 1 s.d. of the calculated trends in differences for the NOAA sites.

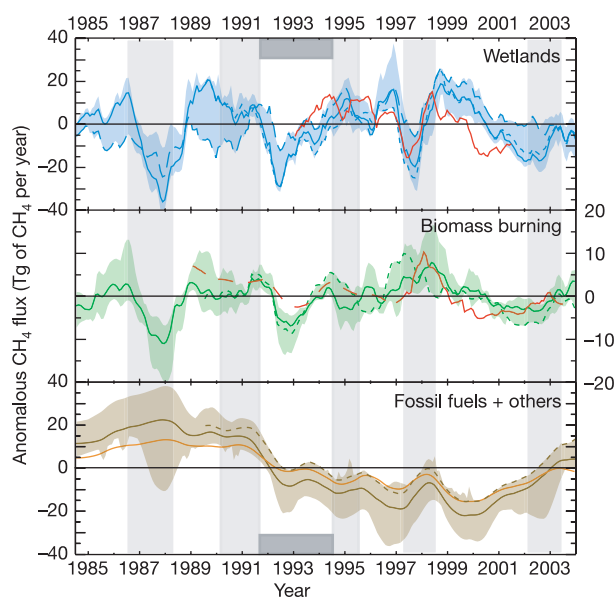


Figure 2 | Variations in CH_4 emissions attributed to different processes. Shown are the interannual global CH_4 flux anomalies (in Tg of $\text{CH}_4 \text{ yr}^{-1}$; note different y-axis scales) broken down into different processes. Unbroken lines of each sub-panel indicate the member of the ensemble with only CH_4 observations (control inversion, 68 sites); broken lines of each sub-panel indicate the member of the ensemble with both CH_4 observations (68 sites) and $\delta^{13}\text{C-CH}_4$ observations (13 sites after 1998; 4 sites after 1989). Blue indicates wetlands (including rice agriculture); dashed blue line represents wetland anomaly inferred in the extreme case where OH is maintained constant from year to year. Green indicates biomass burning. Brown indicates energy-related sources (fossil fuels, industry, bio-fuels) and other sources (landfills and waste, ruminants, termites, ocean, plants). Unbroken orange line represents the specific contribution of fossil-fuel emissions alone. Red lines indicate set of bottom-up estimates of CH_4 flux anomalies obtained from a wetland flux model driven by remotely sensed flooded area data²², and from a fire model driven by remote sensing measurements after 1997 (ref. 9), and extrapolated using atmospheric carbon monoxide trends before that date (see Supplementary Information). The anomalies are calculated by subtracting the long-term mean CH_4 flux over the whole period (1984–2003) from the deseasonalized (12-month running mean) monthly flux in each region. Shaded areas represent the spread of an ensemble of 18 inversions (each using *a priori* OH fields pre-optimized from methyl chloroform).

place a strong (tropical) release of CH_4 by fires in 1997, six months earlier than inferred from the remote sensing data (Fig. 2).

The regional patterns of surface CH_4 emissions indicate that most of the global year-to-year variability lies in the tropics (Fig. 3). By contrast, the northern regions show smoother variations, but with systematically less emissions in the 1990s than in the 1980s, except for 1997–1998 and 2002–2003, consistent with Fig. 1b. The variability in CH_4 removal by OH radicals¹⁹ is also dominated by the tropics, where photochemistry remains active all year (Fig. 3). We found that the years 1987–1988, 1991–1992, 1997–1998 and 2001–2002 correspond to abnormally weaker CH_4 destruction by OH in the tropics. In the inversion, a compensation effect exists between the magnitude of methyl-chloroform-derived changes in OH and inverted CH_4 surface emissions, because the sum of the two must equal the observed atmospheric accumulation. Thus, biases in OH changes

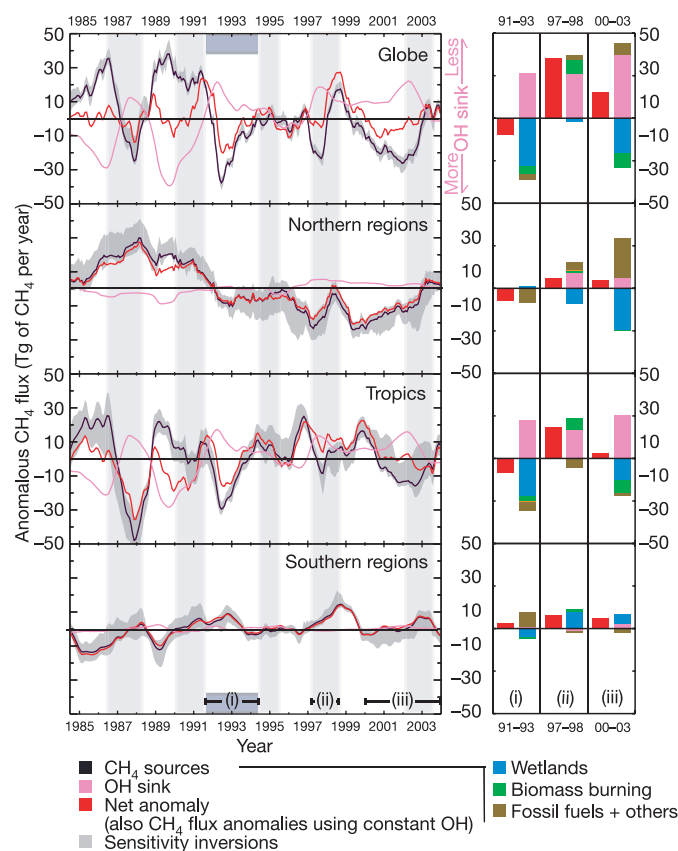


Figure 3 | Large-scale regional variations in CH_4 emissions and OH sink. Left, interannual flux anomalies (in $\text{Tg of CH}_4 \text{ yr}^{-1}$) broken down into three large regions²⁷ for the control inversion: northern regions, tropics and southern regions. Black indicates surface emission anomaly, with grey uncertainty estimates based on the spread of the ensemble of 18 inversions (each using *a priori* OH variations pre-optimized from methyl chloroform). Pink indicates anomalies in CH_4 removal by OH (negative values mean more CH_4 removal). Red indicates anomalies in the net CH_4 budget in each band; that is, the sum of surface emissions and OH removal. This red line also represents what the surface emissions anomalies would be in the extreme case where OH is maintained constant from year to year. Right, changes in surface emissions and in the OH removal term within each large region during selected remarkable episodes analysed in the text: the drop in CH_4 growth rate of 1991–1993; the high CH_4 growth rate of the 1997–1998 largest El Niño event; and the persistent low mean atmospheric growth rate of 2000–2003. The 1991–1993 anomaly is discussed after subtraction of the mean flux over 1984–2003. The 1997–1998 and the 2000–2003 anomalies are discussed after subtraction of the mean flux over 1993–2003. Quantities refer to the full episode (Tg of CH_4). Each source is coloured as in Fig. 2. A positive bar indicates an increase in surface emission and a decrease in OH removal.

could account for some of the variability that we attributed to wetlands. In the extreme case where OH interannual variability is set at zero, the fluctuations of tropical wetland emissions are dampened by 50%, especially in the 1980s, when methyl chloroform data suggest large OH variability¹⁹.

We analysed in detail two key perturbations of the CH_4 budget in the past two decades (Fig. 3). First, we studied the drop in growth rate in 1991–1993. This period is particularly intriguing because of the potentially confounding effects of three factors: (1) reduced photochemical production caused by changes in ultraviolet radiation associated with volcanic aerosols emitted in the eruption of the Mount Pinatubo in June 1991; (2) the widespread Northern Hemisphere cooling that followed²⁵; and (3) the economic collapse of the former Soviet Union. The first factor should decrease OH, causing CH_4 to increase. Indeed, we inferred a decrease in tropical OH by 5% from the methyl chloroform data¹⁹, as suggested by Fig. 1b and previous studies⁴. The two other factors should reduce wetland and fossil-fuel emissions respectively, causing atmospheric CH_4 to decrease. Overall, we attribute the 1991–1993 spike in growth rate, a -10 Tg of CH_4 event (Fig. 3), to a large decrease in emissions ($-36 \pm 6 \text{ Tg of CH}_4$) partly offset by a reduction in the OH sink intensity ($+26 \text{ Tg of CH}_4$). Sources that were reduced in that period are predominantly northern and tropical wetlands ($-24 \pm 6 \text{ Tg of CH}_4$) and anthropogenic sources ($-10 \pm 5 \text{ Tg of CH}_4$). Decreased biomass-burning emissions had a much smaller role ($-5 \pm 2 \text{ Tg of CH}_4$). This inversion result agrees well with an independent study showing reduced boreal wetland emissions due to cooler and dryer conditions¹⁰, and reduced northern anthropogenic emissions²⁶.

Second, we investigated the period 1997–1998, which corresponds to the largest El Niño on record. At that time, widespread dryness caused increases of fires in the tropical zone and in boreal regions of Eurasia⁹. In particular, large abnormal peat fires in Indonesia could have released huge amounts of CH_4 to the atmosphere from smouldering combustion¹⁵. A previous study⁹ estimated an anomalous fire source of $+11.5 \text{ Tg of CH}_4$ in 1997–1998 (Fig. 3), which agrees well with the inversion estimates ($+8 \pm 2 \text{ Tg of CH}_4$ in the tropics and $+2 \pm 1 \text{ Tg of CH}_4$ in northern regions). A larger decrease in OH concentration, possibly also caused by large emissions of carbon monoxide¹⁶ and other reactive carbon compounds by fires⁷, is found to contribute to a faster growth of CH_4 by an additional $+26 \text{ Tg of CH}_4$. Natural wetland emissions remained on average stable over the whole 1997–1998 period. However, there was a significant dip in 1997 for the northern regions wetlands ($-9 \pm 5 \text{ Tg of CH}_4$), followed by an increase in 1998 ($+10 \pm 5 \text{ Tg of CH}_4$) in the southern regions (Fig. 3). This shift in regional wetland emissions is fully consistent with the succession of regionally dryer and wetter climate conditions¹⁸.

Finally, we analysed why the global growth rate of atmospheric CH_4 remained low after the drop in 1991–1993 (ref. 4). After 1993, decreasing global emissions at a rate of $-1.0 \pm 0.2 \text{ Tg of CH}_4 \text{ yr}^{-1}$ are required to match a small average growth rate of $+4 \pm 4 \text{ p.p.b. yr}^{-1}$, in the presence of (slightly) decreasing OH (Fig. 3). The inversion attributes this signal to decreasing anthropogenic emissions, in particular to the northern fossil source (Fig. 3). This is in qualitative agreement with the latitudinal CH_4 differences analysed in Fig. 1b. After 1999, however, anthropogenic emissions increase again, especially in north Asia. This may reflect the booming Chinese economy. By 2003, we find that anthropogenic emissions recovered to their levels in the early 1990s. Without a coincident and important drop in northern wetland emissions after 1999 (Fig. 2) associated with dryer conditions²⁴, the growth rate of atmospheric CH_4 would therefore have increased much more rapidly. This suggests that the slow-down in CH_4 growth rate observed from the early 1990s may represent only a temporary pause in the human-induced secular increase in atmospheric CH_4 .

Better knowledge of the current CH_4 budget helps to reduce uncertainties in future projections of climate change and tropospheric

ozone evolution and to design effective mitigation strategies. Atmospheric long-term measurements and inverse models currently provide key information for assessing CH₄ emission trends at the global to subcontinental scale. Given uncertainties in surface emissions and OH distribution, however, using this approach at the regional or country scale remains challenging and requires an observational network that is dense in space and time²⁷. In the future, the combined use of improved emission inventories, isotopic observations and global space-borne measurements of column-integrated CH₄ should help better to quantify regional sources, to separate natural from anthropogenic processes, and to verify the effectiveness of CH₄ mitigation policies.

METHODS

Inversion model setup. The inverse methodology has been fully described¹⁹ through the example of OH field optimization against methyl chloroform observations. For CH₄, surface emissions are optimized each month for 11 land regions (those defined in ref. 28), one global ocean region, and up to ten processes over each land region (emissions from bogs, swamps, tundra, termites, fossil fuel and industry, gas, bio-fuel, ruminant animals, landfills and waste, and soil uptake). This spatial partition enables us to perform both geographically based and process-based analyses. On a geographical basis, the optimized emissions were further aggregated, after inversion, over three large regions: northern regions (boreal & temperate North America, boreal & temperate Asia, and Europe, roughly >30°N), tropical regions (tropical America, north and South Africa, tropical Asia), and southern regions (temperate South America and Oceania, roughly <30°S). We used the optimized interannual four-dimensional distribution of OH from ref. 19. The variations in OH are pre-optimized from methyl chloroform data using interannual winds and chemistry (see Supplementary Information). In this procedure, the inferred interannual OH fields also depend on methyl chloroform sources. Uptake of CH₄ by soils is optimized as an independent sink, but we do not explicitly solve for the stratospheric sink of CH₄ but assume that it is included in the removal by the stratospheric OH radicals of the INCA model¹⁷.

Atmospheric CH₄ observations, from roughly weekly air samples collected in flasks, were inverted as monthly means. Uncertainties in the monthly means were taken from the GLOBALVIEW-CH₄ data product²⁹, when available, or from submonthly variability in the measurements. In total, data from 68 sites from different networks were collected and used; 75% was contributed by the NOAA network. Offsets between different observing networks were accounted for by using intercomparison round-robin information reported in GLOBALVIEW-CH₄. The sampling periods for each site, and data uncertainties, are given in Supplementary Table A2. Atmospheric δ¹³C-CH₄ flask data from 13 NOAA sites were used in the inversion for the 1998–2004 period (Supplementary Table A2). The δ¹³C-CH₄ values were measured by INSTAAR at the University of Colorado³⁰. At four sites (Point Barrow, Mauna Loa, Samoa and South Pole), the NOAA observations were merged with those from the SIL network³¹ to extend the time series for the period 1989–2004. The gap in δ¹³C-CH₄ data in the period with no observations in 1996–1997 was filled by interpolation, and the interpolated values associated with a large *a priori* uncertainty in the inversion. At Niwot Ridge, the UCI network time-series³² was used to extend the atmospheric δ¹³C-CH₄ record back to 1994. Although isotopic ratios are monitored at only 13 sites, they are expected to constrain usefully the partitioning of CH₄ sources according to their mean isotopic signature: biomass burning (about –20‰ for C-3 plants; about –12‰ for C-4 plants), all bacterial processes (about –60‰) and fossil-fuel-related sources (about –40‰), the atmosphere being close to –47‰ on average. The carbon isotopes measurements are relative to Vienna Pee Dee Belemnite (VPDB).

The inversion of CH₄ fluxes accounts for the fact that CH₄ removal by OH radicals is a nonlinear function of surface CH₄ emissions, by iteratively applying the forward and the inverse transport chemistry model up until convergence is reached for the OH removal of CH₄. In inversions using δ¹³C-CH₄ data, we account for the fact that transport and chemistry of δ¹³C-CH₄ is nonlinear by solving iteratively for both the underlying CH₄ source magnitude and its isotopic composition, as in ref. 33.

Sensitivity tests. The settings of the inversion model that were varied in the sensitivity tests were (1) the *a priori* error on regional fluxes; (2) the *a priori* error on the atmospheric CH₄ and δ¹³C-CH₄ measurements; (3) the number of land regions to be optimized; (4) the size of the atmospheric network; (5) the use of non interannual transport; (6) the uses of non-interannual OH; (7) the introduction of an additional source due to possible CH₄ emission by plants²². See Supplementary Table A2 for a complete description of the 18 inversions performed.

NSD. The normalized standard deviation (NSD) is calculated as the ratio between the s.d. of the monthly deseasonalized inverted CH₄ flux anomaly and the s.d. of the same anomaly calculated by the bottom-up model. An NSD value of 1 indicates a similar variability between the inversion and the bottom-up model.

Received 10 April; accepted 3 August 2006.

1. IPCC. *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change* (eds Houghton, J. T. et al.) (Cambridge Univ. Press, Cambridge and New York, 2001).
2. Dlugokencky, E. J. et al. Atmospheric methane levels off: temporary pause or a new steady-state? *Geophys. Res. Lett.* **30**, 1992, doi:10.1029/2003GL018126 (2003).
3. Dentener, F. et al. Interannual variability and trend of CH₄ lifetime as a measure for OH changes in the 1979–1993 time period. *J. Geophys. Res.* **108**, 4442, doi:10.1029/2002JD002916 (2003).
4. Dlugokencky, E. J. et al. Changes in CH₄ and CO growth rates after the eruption of Mt Pinatubo and their link with changes in tropical tropospheric UV flux. *Geophys. Res. Lett.* **23**, 2761–2764 (1996).
5. Dlugokencky, E. J. et al. A dramatic decrease in the growth-rate of atmospheric methane in the Northern-Hemisphere during 1992. *Geophys. Res. Lett.* **21**, 507–507 (1994).
6. Langenfelds, R. L. et al. Interannual growth rate variations of atmospheric CO₂ and its δ C-13, H-2, CH₄, and CO between 1992 and 1999 linked to biomass burning. *Glob. Biogeochem. Cycles* **16**, 1048, doi:10.1029/2001GB001466 (2002).
7. Manning, M. R., Lowe, D. C., Moss, R. C., Bodeker, G. E. & Allan, W. Short-term variations in the oxidizing power of the atmosphere. *Nature* **436**, 1001–1004 (2005).
8. Page, S. E. et al. The amount of carbon released from peat and forest fires in Indonesia during 1997. *Nature* **420**, 61–65 (2002).
9. Van der Werf, G. R. et al. Continental-scale partitioning of fire emissions during the 1997 to 2001 El Niño/La Niña period. *Science* **303**, 73–76 (2004).
10. Walter, B. P., Heimann, M. & Matthews, E. Modeling modern methane emissions from natural wetlands 2. Interannual variations 1982–1993. *J. Geophys. Res.* **106**, 34207–34219 (2001).
11. Wang, J. S. et al. A 3-D model analysis of the slowdown and interannual variability in the methane growth rate from 1988 to 1997. *Glob. Biogeochem. Cycles* **18**, 3011, doi:10.1029/2003GB002180 (2004).
12. Warwick, N. J., Bekki, S., Law, K. S., Nisbet, E. G. & Pyle, J. A. The impact of meteorology on the interannual growth rate of atmospheric methane. *Geophys. Res. Lett.* **29**, 1947, doi:10.1029/2002GL015282 (2002).
13. Chen, Y. H. & Prinn, R. G. Estimation of atmospheric methane emissions between 1996 and 2001 using a three-dimensional global chemical transport model. *J. Geophys. Res.* **111**, doi:10.1029/2005JD006058 (2006).
14. Mikaloff Fletcher, S. E. M., Tans, P. P., Bruhwiler, L. M., Miller, J. B. & Heimann, M. CH₄ sources estimated from atmospheric observations of CH₄ and its C-13/C-12 isotopic ratios: 1. Inverse modeling of source processes. *Glob. Biogeochem. Cycles* **18**, GB4004, doi:10.1029/2004GB002223 (2004).
15. Simmonds, P. G. et al. A burning question. Can recent growth rate anomalies in the greenhouse gases be attributed to large-scale biomass burning events? *Atmos. Environ.* **39**, 2513–2517 (2005).
16. Butler, T. M., Rayner, P. J., Simmonds, I. & Lawrence, M. G. Simultaneous mass balance inverse modeling of methane and carbon monoxide. *J. Geophys. Res.* **110**, D21310, doi:10.1029/2005JD006071 (2005).
17. Hauglustaine, D. A. et al. Interactive chemistry in the Laboratoire de Meteorologie Dynamique general circulation model: description and background tropospheric chemistry evaluation. *J. Geophys. Res.* **109**, D04314, doi:10.1029/2003JD003957 (2004).
18. Uppala, S. M. et al. The ERA-40 reanalysis. *Q. J. R. Meteorol. Soc.* **131**, 2961–3012 (2005).
19. Bousquet, P., Hauglustaine, D. A., Peylin, P., Carouge, C. & Ciais, P. Two decades of OH variability as inferred by an inversion of atmospheric transport and chemistry of methyl chloroform. *Atmos. Chem. Phys.* **5**, 2635–2656 (2005).
20. Bousquet, P. et al. Regional changes in carbon dioxide fluxes of land and oceans since 1980. *Science* **290**, 1342–1346 (2000).
21. Rodenbeck, C., Houweling, S., Gloor, M. & Heimann, M. CO₂ flux history 1982–2001 inferred from atmospheric data using a global inversion of atmospheric transport. *Atmos. Chem. Phys.* **3**, 1919–1964 (2003).
22. Keppler, F., Hamilton, J. T. G., Braß, M. & Rockmann, T. Methane emissions from terrestrial plants under aerobic conditions. *Nature* **439**, 187–191 (2006).
23. Prigent, C., Matthews, E., Aires, F. & Rossow, W. B. Remote sensing of global wetland dynamics with multiple satellite data sets. *Geophys. Res. Lett.* **28**, 4631–4634, doi:10.1029/2001GL013263 (2001).
24. Hoerling, M. & Kumar, A. The perfect ocean for drought. *Science* **299**, 691–694 (2003).
25. Hansen, J., Ruedy, R., Sato, M. & Reynolds, R. Global surface air temperature in 1995: return to pre-Pinatubo level. *Geophys. Res. Lett.* **23**, 1665–1668 (1996).
26. Dlugokencky, E. J., Steele, L. P., Lang, P. M. & Masarie, K. A. The growth-rate and distribution of atmospheric methane. *J. Geophys. Res.* **99**, 17021–17043 (1994).

27. Bergamaschi, P. *et al.* Inverse modelling of national and European CH₄ emissions using the atmospheric zoom model TM5. *Atmos. Chem. Phys.* **5**, 2431–2460 (2005).
28. Gurney, K. R. *et al.* Towards robust regional estimates of CO₂ sources and sinks using atmospheric transport models. *Nature* **415**, 626–630 (2002).
29. GLOBALVIEW-CH₄. *Cooperative Atmospheric Data Integration Project—Methane* CD-ROM (NOAA/CMDL, Boulder, CO, 2005).
30. Miller, J. B. *et al.* Development of analytical methods and measurements of C-13/C-12 in atmospheric CH₄ from the NOAA Climate Monitoring and Diagnostics Laboratory global air sampling network. *J. Geophys. Res.* **107**, 4178, doi:10.1029/2001JD000630 (2002).
31. Quay, P. *et al.* The isotopic composition of atmospheric methane. *Glob. Biogeochem. Cycles* **13**, 445–461 (1999).
32. Tyler, S. C. *et al.* Stable carbon isotopic composition of atmospheric methane: a comparison of surface level and free tropospheric air. *J. Geophys. Res.* **104**, 13895–13910 (1999).
33. Hein, R., Crutzen, P. J. & Heimann, M. An inverse modeling approach to investigate the global atmospheric methane cycle. *Glob. Biogeochem. Cycles* **11**, 43–76 (1997).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank P. Rayner, F. Chevallier and F.-M. Breon for comments on the manuscript, and P. Quay for published $\delta^{13}\text{C}$ -CH₄

measurements for the period 1989–1995. Atmospheric CH₄ measurements from Réseau Atmosphérique de Mesure des Composés à Effet de Serre (RAMCES) at Laboratoire des Sciences du Climat et de l'Environnement (LSCE) were partly funded by Institut National des Sciences de l'Univers (INSU). All calculations were realized with Commissariat à l'Energie Atomique (CEA), Centre National de la Recherche Scientifique (CNRS), Institut Pierre Simon Laplace (IPSL) and LSCE computers and support. The development of the Global Fire Emissions Dataset (GFED) used here was supported by a grant from the National Aeronautics and Space Administration (NASA).

Author Contributions The main contributions of each author are: P.B.: inversions, data analysis and coordination. P.C.: inverse method and manuscript improvements. J.B.M.: CH₄ and $\delta^{13}\text{C}$ -CH₄ data analysis and inversion analysis. E.J.D.: CH₄ measurements and manuscript improvements. D.A.H.: chemistry-transport model and manuscript improvements. C.P. and F.P.: satellite retrievals of flooded areas. G.R.V.d.W.: CH₄ emissions from fires. P.P. and C.C.: inversion method. R.L.L.: CH₄ measurements and manuscript improvements. E.G.B., M.R., M.S., L.P.S. and S.C.T.: CH₄ measurements. J.L.: plant source analysis. J.W.: $\delta^{13}\text{C}$ -CH₄ measurements.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to P.B. (philippe.bousquet@cea.fr).

LETTERS

Continental-scale patterns of canopy tree composition and function across Amazonia

Hans ter Steege¹, Nigel C. A. Pitman², Oliver L. Phillips³, Jerome Chave⁴, Daniel Sabatier⁵, Alvaro Duque⁶, Jean-François Molino⁵, Marie-Françoise Prévost⁷, Rodolphe Spichiger⁸, Hernán Castellanos⁹, Patricio von Hildebrand¹⁰ & Rodolfo Vásquez¹¹

The world's greatest terrestrial stores of biodiversity and carbon are found in the forests of northern South America, where large-scale biogeographic patterns and processes have recently begun to be described^{1–4}. Seven of the nine countries with territory in the Amazon basin and the Guiana shield have carried out large-scale forest inventories, but such massive data sets have been little exploited by tropical plant ecologists^{5–8}. Although forest inventories often lack the species-level identifications favoured by tropical plant ecologists, their consistency of measurement and vast spatial coverage make them ideally suited for numerical analyses at large scales, and a valuable resource to describe the still poorly understood spatial variation of biomass, diversity, community composition and forest functioning across the South American tropics⁹. Here we show, by using the seven forest inventories complemented with trait and inventory data collected elsewhere, two dominant gradients in tree composition and function across the Amazon, one paralleling a major gradient in soil fertility and the other paralleling a gradient in dry season length. The data set also indicates that the dominance of Fabaceae in the Guiana shield is not necessarily the result of root adaptations to poor soils (nodulation or ectomycorrhizal associations) but perhaps also the result of their remarkably high seed mass there as a potential adaptation to low rates of disturbance.

The trees in our combined database number more than a quarter of a million (277,069). Nearly 40% of these are from the 1968–78 RADAMBRASIL surveys of the Brazilian Amazon¹⁰. The data set currently includes 89 families and 513 genera. The ten most common families in order of abundance are Fabaceae (legumes), Sapotaceae, Lecythidaceae, Moraceae, Burseraceae, Chrysobalanaceae, Malvaceae, Euphorbiaceae, Lauraceae and Myristicaceae; these account for 74% of all trees. One-quarter of all large trees in the data set belong to the Fabaceae. The ten most common genera in order of abundance are *Eschweilera* (5.6%), *Pouteria*, *Licania*, *Tetragastris*, *Eperua*, *Inga*, *Protium*, *Swartzia* and *Virola*.

Mapping the results of a multivariate ordination of the genus-level compositional data reveals two primary and independent geographical gradients in tree community composition, which together explain 24% of all variance. One gradient stretches from the Guiana shield to southwestern Amazonia (Fig. 1a and Supplementary Fig. S1) and the other from Colombia to southeastern Amazonia (Fig. 1b). The first gradient reflects the fact that none of the ten most abundant genera in

the Guiana shield area (*Carapa*, *Lecythis*, *Aldina*, *Pentaclethra*, *Alexa*, *Dicorynia*, *Eperua*, *Catostemma*, *Mora* and *Dicymbe*) are among the ten most abundant genera (or at all) in western Amazonia (*Iriarte*, *Attalea*, *Otoba*, *Oenocarpus*, *Pseudolmedia*, *Ficus*, *Clarisia*, *Sapium*, *Spondias* and *Cecropia*), as well as the fact that seven of the ten top genera of the Guiana shield, but none of the top ten western Amazonian genera, are legumes. This first gradient in large-canopy trees agrees with earlier findings based on smaller trees and analysed at the family level^{1,2}. The second gradient, northwest to southeast, is characterized by an increasing abundance of Burseraceae (*Protium* and *Tetragastris*), Bignoniaceae (*Jacaranda* and *Tabebuia*) and Rutaceae (*Fagara*) in drier forests at the southeastern margin of the Amazon basin.

The first gradient is congruent with parallel gradients in community-averaged wood density and seed mass (Figs 1c, d and 2a, b), and regional soil fertility (Fig. 2c). At the Guiana shield end of the gradient, soils are poorer, wood is denser, and seeds are larger. The latter life-history characteristics are important functional traits that can predict species behaviour under various disturbance regimes. Seed mass can be regarded as an indicator of the type of establishment^{9,11}: species with small seeds germinate and grow fast in large gaps, whereas species with large seeds mainly survive and grow (slowly) in smaller gaps or the shaded understory. In other words, we consider average community seed mass to be an indicator of the level of landscape level disturbance⁹. Wood density can be regarded as an indicator of adult tree growth and survival^{9,11}: models show that species with heavy wood are less competitive in more dynamic forests^{9,11}. Because average wood density in a community is also expected to covary with long-term forest disturbance⁹, our data indicate that long-term disturbance regimes might be lower on the geologically older and poorer Guiana shield than on the recently deposited sediments of western Amazonia. It has indeed been shown that higher soil fertility leads to higher productivity and, consequently, higher turnover of individuals^{3,4}. The correspondence of these gradients to the gradient of tree diversity in the region (Fig. 1h; R^2 axis 1 score with Fisher's $\alpha = 0.35$) thus provides support for the intermediate disturbance hypothesis¹², which predicts that low disturbance rates lead to competitive exclusion and depress diversity. Indeed, mono-dominance is also strongly related to the first compositional gradient ($R^2 = 0.32$; data not shown).

The second major gradient is strongly related to dry season length

¹Institute of Environmental Biology, Section Plant Ecology and Biodiversity, and the National Herbarium of the Netherlands NHN, Utrecht University branch, Sorbonnelaan 14–16, 3584 CA Utrecht, The Netherlands. ²Amazon Conservation Association, 1731 Connecticut Avenue NW 3rd floor, Washington DC 20009, USA. ³Earth and Biosphere Institute, School of Geography, University of Leeds, Leeds LS2 9JT, UK. ⁴CNRS/UPS, Évolution et Diversité Biologique, UMR 5174, UPS Toulouse III, Bâtiment IVR3, F-31062 Toulouse, France. ⁵Institut de Recherche pour le Développement (IRD), UMR AMAP, TA40/PS2, 34398 Montpellier cedex 5, France. ⁶Universidad Nacional de Colombia, Departamento de Ciencias Forestales, Calle 64 x Carrera 65, A.A. 1027, Medellín, Colombia. ⁷Institut de Recherche pour le Développement (IRD), UMR AMAP, BP 165, 97323 Cayenne cedex, French Guiana. ⁸Conservatoire et Jardin Botaniques de la Ville de Genève, CP 60, CH-1292 Chambésy, Genève, Switzerland. ⁹UNEG, Calle Chile, Urbaniz Chilemex, Puerto Ordaz, Edo. Bolívar, Venezuela. ¹⁰Fundación Puerto Rastrojo, Cra. no. 24–76 of. 1201, Bogotá DC A.A. 241438, Colombia. ¹¹Proyecto Flora del Perú, Jardín Botánico de Missouri, Jaen, Cajamarca, Peru.

(DSL; Fig. 2d). DSL is known to limit maximum tree α -diversity across the South American tropics¹³ (in the current data set, DSL also affects Fisher's α in this manner). The forest inventory data confirm predictions that it also has a major effect on geographic variation in tree community composition across the Amazon basin.

The dominant floristic gradient probably predates the Quaternary period, because the underlying fertility gradient reflects a deep-seated contrast between slow weathering of the quartz-rich Precambrian Guyanan and Brazilian shields in the east, and rapid weathering and deposition of new materials from the Andes in the west, for more than 20 Myr (ref. 14). By contrast, the second gradient may be more dynamic, to the extent that the relative performance of species tracks the constantly shifting Cenozoic climate¹⁵. Thus, the decreased precipitation and increased temperature predicted for the Amazon basin in some climate change models¹⁶ could result in significant shifts in tree composition along this gradient within human timescales.

Because the first gradient is so strongly associated with the abundance of legumes (Fabaceae; Fig. 1f), we investigated the geographical distribution of two characteristics commonly associated with the

family: nitrogen-fixing nodulation and ectomycorrhizal associations, both assumed adaptations to low-fertility soils¹⁷. Ectomycorrhizal association is apparently insignificant in northern South America (Fig. 1e), apart from small areas in Guyana (where the ectomycorrhizal genus *Dicymbe* is common) and the upper Rio Negro (where the ectomycorrhizal genus *Aldina* is common). Not surprisingly, individuals of nodulating genera are more common on poor soils (Figs 1g and 2e). However, individuals of nodulating genera represent a smaller fraction of all Fabaceae on poor soils than they do on rich soils (Fig. 2f), and nodulation within Fabaceae is strongly negatively correlated with dominance of Fabaceae (Fig. 2g), indicating that some other factor might be responsible for the dominance of legumes in South American forests on poor soils.

One such factor may be seed mass. As shown in Fig. 1c, average community seed mass increases by more than an order of magnitude along the main compositional gradient from the southwest Amazonia to central Guyana; in addition, wood density increases along this gradient. The increase of community seed mass is driven by an increase in seed mass of both legumes ($R^2 = 0.81$) and non-legumes ($R^2 = 0.66$). However, the average seed mass of legume trees

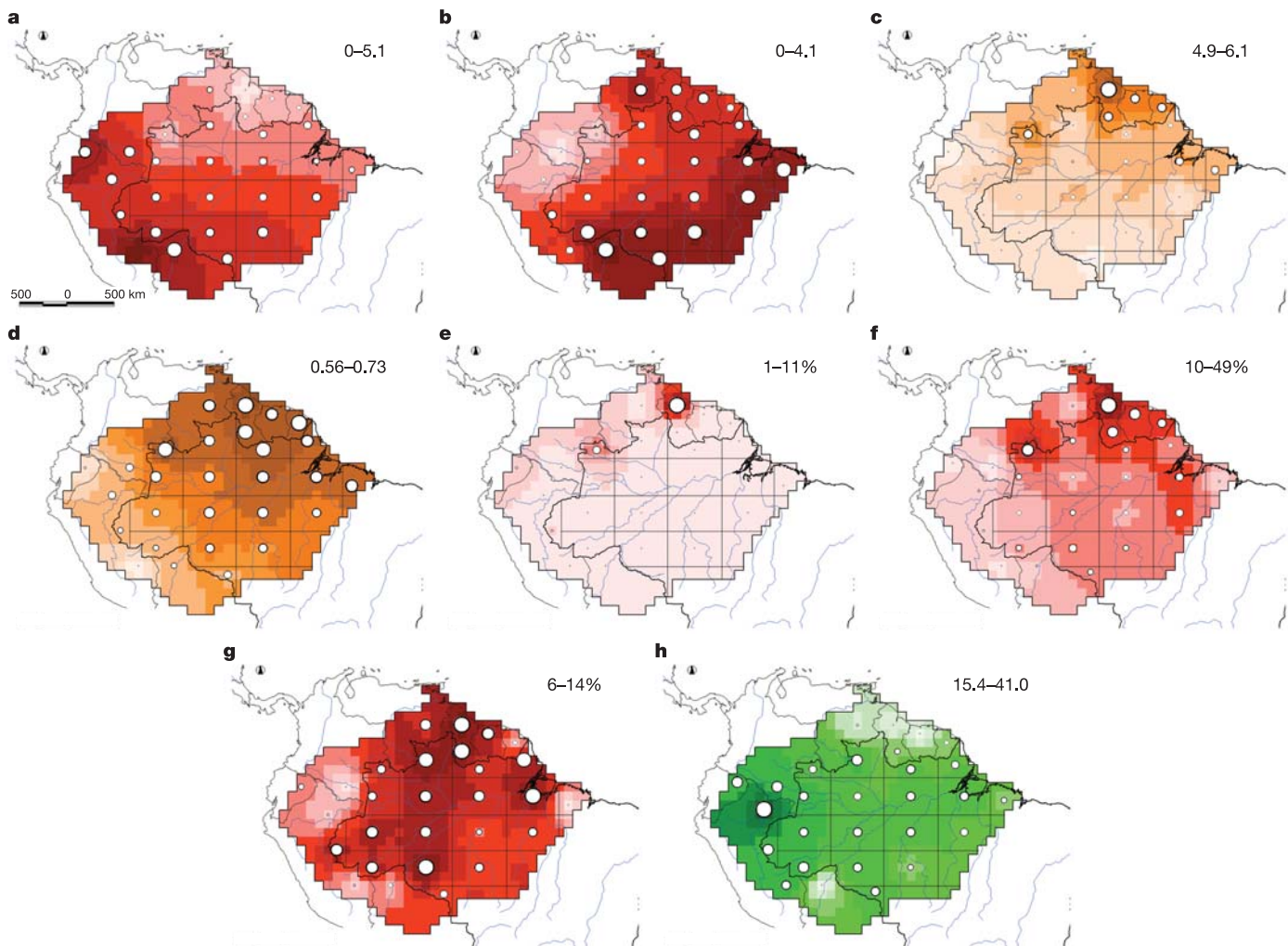


Figure 1 | Geographic variation in community characteristics of South American tree communities. Values in each region are illustrated by the sizes of the open circles and are based on all individuals in that region. Numbers in the top right corners indicate minimum and maximum values. Colours represent an interpolation of the same data by inverse distance weighting on a 1° grid; darker colours indicate higher values. The distance scale in **a** applies to all panels. **a**, Scores on the first axis of the gradient analysis (detrended correspondence analysis) of genus-level community

composition. **b**, Scores on the second axis of the same analysis. **c**, Community-weighted seed mass in logarithmic classes (see Methods). **d**, Community-weighted wood density (oven-dried weight divided by green volume). **e**, Proportion of all trees belonging to ectomycorrhizal genera. **f**, Proportion of all trees belonging to Fabaceae. **g**, Proportion of all trees belonging to nodulating genera. **h**, Large tree diversity, calculated with Fisher's α index from the number of individuals and number of genera in each region.

increases more than that of non-legumes (Fig. 2h). The higher wood density in eastern Amazonia and the Guiana shield is also explained by an increase in wood density within Fabaceae ($R^2 = 0.85$) and non-Fabaceae ($R^2 = 0.27$) but their relative increase is identical (Fig. 2h) when plotted against Fabaceae abundance. Thus, on the richer soils of western Amazonia the average seed masses of Fabaceae and non-Fabaceae are similar, whereas on the poor soils of the Guiana shield Fabaceae have a 20% higher average seed mass. This indicates that Fabaceae might be successful in low-dynamics environments because Fabaceae species are better at producing large seeds and, subsequently, more shade-tolerant seedlings, than other families. Janzen¹⁸ has linked the high seed mass of legumes to their relative success on poor soils and correctly predicted that their seeds should be very poisonous to protect them from excessive predation¹⁹.

METHODS

Data gathering. Data were gathered from large-scale national resource inventories, often initiated by the United Nations Food and Agriculture Organization's Forestry Programme. The minimum diameter of the trees recorded was 30 cm, so our analysis is effectively confined to canopy trees. In cases where resource inventories were not available, incomplete, or too small to be representative, they were replaced or supplemented by the large trees of 1-ha inventories (see Supplementary Information).

Harmonizing names and taxonomy between the inventories. Vernacular names in the forest inventory data sets were converted to genus-level scientific names with the lists included in the references used (Supplementary Information). We conducted the analysis at the genus level for three reasons. First, vernacular names are often unreliable at the species level but accurate at the genus level. We estimate that more than 95% of individuals are correctly identified to genus^{6,20}. Even so, we quantified the effect of potential 'translation' errors from the vernacular to the genus level by also performing an analysis at the family level (Supplementary Information). Second, because of incomplete sampling and because some species have small range sizes, many species will have been reported from only one inventory location, so that noise in a species-level analysis would overwhelm any underlying regional signal in floristic variation. Third, most species within a genus have similar values for the life-history characteristics we studied^{21,22}. Analysis at the generic level thus reduces error and noise in compositional patterns, while not unduly sacrificing the subtlety of signal detectable in life-history characteristics. At the time of the analysis all trees in the database had been classified to family, and more than 98% classified to genus.

All genus names were checked with a variety of sources, notably *w*³Tropicos (<http://mobot.mobot.org/W3T/Search/vast.html>), The International Plant Names Index (<http://www.ipni.org/index.html>) and the Legume Web of the International Legume Database and Information Service (<http://www.ildis.org/LegumeWeb/>). Taxonomy was standardized to family and genus level as described by the Angiosperm Phylogeny Group²³, because this system offers the best predictability for life-history characteristics²³.

Data analysis. To map compositional patterns (Fig. 1a, b), we first divided the study area into regions, based on the Radambrasil blocks of $4^\circ \times 6^\circ$, and combined all inventories within each region (see Supplementary Information). We converted the number of individuals of each genus in each region to a percentage, to correct for unequal sample sizes. Ordination of all regions was performed with detrended correspondence analysis (DCA; MVSP 3.11; Kovach Computing Services). We used DCA because this method models species occurrence as a unimodal, gaussian, response curve over gradients, just as we expect species to behave. (An ordination with nonmetric multi-dimensional scaling, which does not make this assumption, gave almost identical results; see Supplementary Information).

Life-history characteristics were scored by genus, because most variation is expected above this level (see above). For seed mass (in logarithmic classes: 0, more than 0.00001 to 0.0001 g; 1, more than 0.0001 to 0.001 g; and so on to 7, more than 100 to 1,000 g) we used data from ref. 9. If seed mass data were not available for a given genus in that reference, seed size was taken from refs 24 and

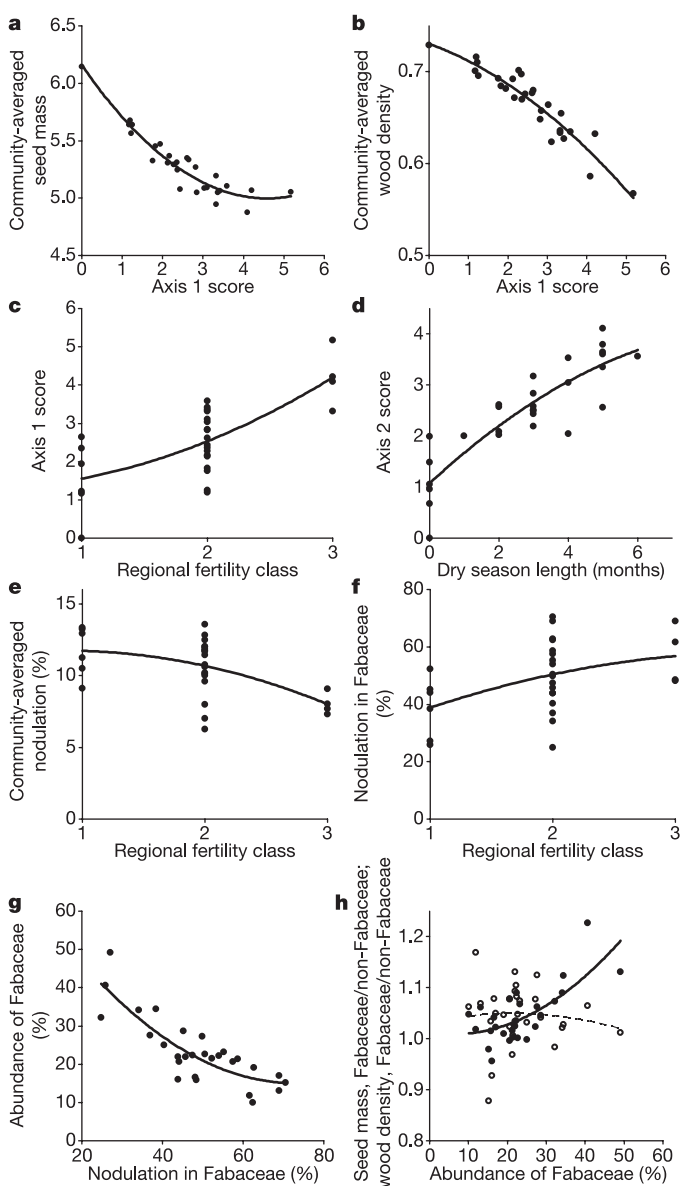


Figure 2 | Relationships between community characteristics of South American tree communities and community characteristics with abiotic factors. **a**, Community-weighted seed mass is strongly correlated with the primary compositional gradient in the study area. Plots on the left with high average seed mass are found in the Guiana shield, whereas plots on the right with low average seed mass are found in western Amazonia. $R^2 = 0.91$. **b**, Community-weighted wood density (oven-dried weight divided by green volume) is strongly correlated with the primary compositional gradient in the study area. Plots on the left with high wood density are found in the Guiana shield, whereas plots on the right with low wood density are found in western Amazonia. $R^2 = 0.88$. **c**, Regional fertility may be one of the factors influencing the primary compositional gradient in the study area. $R^2 = 0.52$. **d**, DSL is correlated with the secondary compositional gradient in the study area. $R^2 = 0.77$. **e**, The proportion of all trees belonging to nodulating genera is higher where soils are poorer. $R^2 = 0.29$. **f**, The proportion of Fabaceae trees belonging to nodulating genera is lower where soils are poorer. $R^2 = 0.21$. **g**, Abundance of Fabaceae (proportion of individuals of the community that belong to Fabaceae) is negatively related to the proportion of individuals of Fabaceae (in the community) with nodulation. $R^2 = 0.71$. **h**, The ratio of seed mass of Fabaceae individuals to non-Fabaceae individuals in Amazonian tree inventories (black circles and solid line) increases with the abundance of Fabaceae in the sample ($R^2 = 0.53$). Where Fabaceae account for less than 20% of trees, the average seed mass of Fabaceae individuals equals that of non-Fabaceae individuals; where Fabaceae account for more than 20% of trees, seed mass of Fabaceae is higher than that of non-Fabaceae. Where the abundance of Fabaceae is high, wood density of both Fabaceae ($R^2 = 0.68$) and non-Fabaceae ($R^2 = 0.40$) is higher but their relative increase is identical (open circles and dotted line; $R^2 = 0.01$).

25, and from local floras and converted to seed mass in accordance with relationships established in ref. 26. Wood density (oven-dried weight divided by green volume²²) was taken from refs 9, 20 and 22. These sources provided seed mass data for 97.1% and wood density data for 97.5% of all individuals in the data set.

Data for nodulation among legumes were taken from ref. 17, which provided information for all except three rare genera. Information for ectomycorrhizal infection was compiled from a variety of references. Only five neotropical tree genera in our database were found to be ectomycorrhizal (*Dicymbe*, *Aldina*, *Neea*, *Pisonia* and *Coccoloba*).

When calculating site scores for the life-history characteristics, all individuals were counted. Thus, the values are not averages based on the number of species but real community averages weighted for all individuals in that community.

DSL (number of months per year averaging less than 100 mm of rainfall) was taken from ref. 13 (based on ref. 27) and averaged over the sub-areas of the 28 forest inventory regions.

Following refs 3 and 4, soil quality was classified into classes based on our field data where available, or else inferred from the landform and geographical context available⁴. The spatial unit used in our floristic analysis was large (more than 25,000 km²), so we used three broad distinctive categories: 1, predominantly poor soil areas (Guianas, Venezuela and upper Rio Negro) with highly oligotrophic spodosols (white sands) and oxisols derived from highly weathered Precambrian sources; 2, edaphically young areas of western Amazonia (Bolivia, Peru, Ecuador and Colombia) with relatively eutrophic inceptisols and ultisols, predominantly derived from Quaternary and Holocene Andean sediment, or Miocene estuarine and marine deposits; and 3, all other areas, with moderately oligotrophic soils, predominantly oxisols derived from Precambrian substrates or weathered Tertiary sediments.

Received 21 June; accepted 3 August 2006.

1. Terborgh, J. & Andresen, E. The composition of Amazonian forests: patterns at local and regional scale. *J. Trop. Ecol.* **14**, 645–664 (1998).
2. ter Steege, H. *et al.* An analysis of the floristic composition and diversity of Amazonian forests including those of the Guiana Shield. *J. Trop. Ecol.* **16**, 801–828 (2000).
3. Phillips, O. L. *et al.* Pattern and process in Amazon tree turnover, 1976–2001. *Phil. Trans. R. Soc. Lond. B* **359**, 381–407 (2004).
4. Malhi, Y. *et al.* The above-ground coarse wood productivity of 104 Neotropical forest plots. *Glob. Change Biol.* **10**, 563–591 (2004).
5. van Rompaey, R. S. A. R. *Forest Gradients in West Africa*. PhD thesis, Wageningen Univ. (1993).
6. ter Steege, H. The use of forest inventory data for a National Protected Area Strategy in Guyana. *Biodivers. Conserv.* **7**, 1457–1483 (1998).
7. ter Steege, H. & Zondervan, G. in *Plant Diversity in Guyana with Recommendations for a National Protected Area Strategy* (ed. ter Steege, H.) 35–54 (Tropenbos Series 18, Tropenbos Foundation, Wageningen, 2000).
8. Bongers, F., Poorter, L. & Hawthorne, W. D. in *Biodiversity of West African Forests. An Ecological Atlas of Woody Plant Species* (eds Poorter, L., Bongers, F., Kouame, F. N. & Hawthorne, W.) 41–52 (CABI Publishing, Wallingford, 2004).
9. ter Steege, H. & Hammond, D. S. Character convergence, diversity, and disturbance in tropical rain forest in Guyana. *Ecology* **82**, 3197–3212 (2001).
10. RADAMBRASIL. *Levantamento de Recursos Naturais* (Ministério de Minas e Energia, Departamento Nacional de Produção Mineral, Rio de Janeiro, 1968–1978).
11. ter Steege, H. *Long-term Changes in Tropical Tree Diversity: Studies from the Guiana Shield, Africa, Borneo and Melanesia* (Tropenbos Series 22, Tropenbos International, Wageningen, 2003).
12. Connell, J. H. Diversity in tropical rain forests and coral reefs. *Science* **199**, 1302–1309 (1978).
13. ter Steege, H. *et al.* A spatial model of tree α -diversity and -density for the Amazon Region. *Biodivers. Conserv.* **12**, 2255–2276 (2003).
14. Gregory-Wodzicki, K. Uplift history of the Central and Northern Andes: a review. *Geol. Soc. Am. Bull.* **112**, 1091–1105 (2000).
15. Bush, M. B., Silman, M. R. & Urrego, D. H. 48,000 years of climate and forest change in a biodiversity hot spot. *Science* **303**, 827–829 (2004).
16. Houghton, J. T. *et al.* *Climate Change 2001: The Scientific Basis* (Cambridge Univ. Press, Cambridge, 2001).
17. Sprent, J. I. *Nodulation in Legumes* (Royal Botanic Gardens, Kew, 2001).
18. Janzen, D. H. Tropical blackwater rivers, animals, and mast fruiting by the dipterocarpaceae. *Biotropica* **6**, 69–103 (1974).
19. Rankin, J. M. *The Influence of Seed Predation and Plant Competition on Three Species Abundances in Two Adjacent Tropical Rain Forest Communities in Trinidad, West Indies*. PhD thesis, Univ. of Michigan (1978).
20. Fearnside, P. M. Wood density for estimating forest biomass in Brazilian Amazonia. *For. Ecol. Manage.* **90**, 59–87 (1997).
21. Casper, B. B., Heard, S. B. & Apanius, V. Ecological correlates of single-seededness in a woody tropical flora. *Oecologia* **90**, 212–217 (1992).
22. Chave, J. *et al.* Regional and phylogenetic variation of wood density across 2456 neotropical tree species. *Ecol. Appl.* (in the press).
23. Stevens, P. F. Angiosperm Phylogeny Website, Version 6, May 2005. (<http://www.mobot.org/MOBOT/research/APweb/>) (2005).
24. van Rosmalen, M. G. M. *Fruits of the Guianan Flora* (Institute of Systematic Botany, Utrecht Univ., Utrecht, 1985).
25. Seed Information Database. *SID release 6.0* (<http://www.rbgekew.org.uk/data/sid/>) (2004).
26. Hammond, D. S. & Brown, V. K. Seed weight of woody plants in relation to disturbance, dispersal, soil type in wet Neotropical forests. *Ecology* **76**, 2544–2561 (1995).
27. Sombroek, W. Spatial and temporal patterns of Amazon rainfall. Consequences for the planning of agricultural occupation and protection of primary forests. *Ambio* **30**, 388–396 (2001).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank the Guyana Forestry Commission, the Center for Agricultural Research in Suriname (CELOS), Cirad-Forêt, Office National des Forêts (ONF) and Aernout Weeda of Zonig for making data available for this research; P. Haripersaud for typing in practically all of the RADAMBRASIL data; and P.-M. Forget, M. Werger, H. During and M. Pascal for discussions and comments on the manuscript. This work would not have been possible without decades of dedicated fieldwork by hundreds of colleagues and forestry workers across South America.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to H.t.S. (h.terstegee@bio.uu.nl).

LETTERS

Increasing $p16^{INK4a}$ expression decreases forebrain progenitors and neurogenesis during ageing

Anna V. Molofsky^{1*}, Shalom G. Slutsky^{1*}, Nancy M. Joseph¹, Shenghui He¹, Ricardo Pardal^{1†}, Janakiraman Krishnamurthy², Norman E. Sharpless² & Sean J. Morrison¹

Mammalian ageing is associated with reduced regenerative capacity in tissues that contain stem cells^{1,2}. It has been proposed that this is at least partially caused by the senescence of progenitors with age^{3,4}; however, it has not yet been tested whether genes associated with senescence functionally contribute to physiological declines in progenitor activity. Here we show that progenitor proliferation in the subventricular zone and neurogenesis in the olfactory bulb, as well as multipotent progenitor frequency and self-renewal potential, all decline with age in the mouse forebrain. These declines in progenitor frequency and function correlate with increased expression of $p16^{INK4a}$, which encodes a cyclin-dependent kinase inhibitor linked to senescence⁵. Ageing $p16^{INK4a}$ -deficient mice showed a significantly smaller decline in subventricular zone proliferation, olfactory bulb neurogenesis, and the frequency and self-renewal potential of multipotent progenitors. $p16^{INK4a}$ deficiency did not detectably affect progenitor function in the dentate gyrus or enteric nervous system, indicating regional differences in the response of neural progenitors to increased $p16^{INK4a}$ expression during ageing. Declining subventricular zone progenitor function and olfactory bulb neurogenesis during ageing are thus caused partly by increasing $p16^{INK4a}$ expression.

Stem cells must persist throughout adult life in numerous tissues, including the central nervous system (CNS)⁶, in order to replace the mature cells that are lost to turnover, injury, or disease. However, the function of stem cells and other progenitors declines with age in diverse tissues including the haematopoietic system^{7–9}, muscle^{10,11} and brain^{6,12,13}. Consistent with this, ageing tissues exhibit reduced repair capacity and an increased incidence of degenerative disease^{1,4}. However, the mechanisms responsible for the age-related decline in the function of stem cells and other progenitors remain uncertain.

$p16^{INK4a}$ gene expression increases with age in a variety of tissues^{14–16}. Although induction of $p16^{INK4a}$ expression can cause the senescence of a variety of cell types in culture^{5,17} and *in vivo*¹⁸, some cells (including some neural progenitors) are unaffected by increased $p16^{INK4a}$ expression or $p16^{INK4a}$ deletion^{19–21}. It is thus unclear whether increased $p16^{INK4a}$ expression causes declines in progenitor function during ageing *in vivo*. We have addressed this question by examining progenitor frequency, proliferation and neurogenesis in the forebrain lateral ventricle subventricular zone (SVZ) of ageing wild-type and $p16^{INK4a}$ -deficient mice. The SVZ contains a mixed population of stem cells and other progenitors that engage in neurogenesis throughout adult life^{6,13,22}. The rate of neurogenesis is known to decline in ageing mammals¹³, but the physiological mechanisms responsible for this decline have not been identified.

We compared various measures of progenitor function in the SVZ

of 60-day-old, 1-yr-old and 2-yr-old mice to determine the effects of ageing. Consistent with a previous study⁶, we found a twofold reduction with age in the frequency of SVZ cells that formed multipotent neurospheres in culture (Fig. 1a; asterisk, $P < 0.05$). This was associated with an approximately twofold reduction in the self-renewal potential of these multipotent neurospheres (Fig. 1b; asterisk, $P < 0.05$). *In vivo*, we observed an approximately threefold reduction in the rate of proliferation in the SVZ with age (Fig. 1c, d; asterisk, $P < 0.05$). These data suggest that stem cell frequency and self-renewal potential, as well as overall proliferation rate, decline with age in the SVZ.

Adult *Bmi1*-deficient mice also exhibit reduced stem cell frequency and self-renewal potential, as well as reduced proliferation in the SVZ²⁰. These effects are largely caused by increased $p16^{INK4a}$ and *Arf* expression in the absence of *Bmi1* (refs 19–21). We therefore wondered whether the age-related changes observed in wild-type mice (Fig. 1) were associated with increased $p16^{INK4a}$ or *Arf* expression in neural progenitors. $p16^{INK4a}$ and *Arf* expression increase with age in some tissues^{15,16}, although these studies did not examine neural progenitors. We did not detect any age-related increase in *Arf* expression in uncultured SVZ cells at the RNA or protein levels (data not shown). In contrast, $p16^{INK4a}$ expression increased substantially with age in uncultured SVZ cells (Fig. 1e). $p16^{INK4a}$ expression was not detectable by polymerase chain reaction (PCR) in the SVZ of 60-day-old mice, but became detectable by 1 yr of age and further increased by 2 yr of age. We were not able to detect $p16^{INK4a}$ protein in the SVZ by western blot, consistent with previous studies that have also not been able to detect this protein in most uncultured mouse tissues that express $p16^{INK4a}$ mRNA^{15,16}, presumably due to its low expression level and the limited sensitivity of available antibodies.

To test the function of $p16^{INK4a}$ in ageing neural progenitors we examined young (60-day-old) and old (2-yr-old) $p16^{INK4a}$ -deficient mice as well as littermate controls. $p16^{INK4a}$ deficiency did not grossly affect the composition of the SVZ, with similar proportions of SVZ cells staining positively for the glia marker GFAP or the neuroblast marker doublecortin in wild-type and $p16^{INK4a}$ -deficient mice irrespective of age (data not shown). Similarly, apoptotic SVZ cells (activated caspase-3⁺) were rare in all treatments (data not shown). Deletion of $p16^{INK4a}$ in young mice did not significantly affect the frequency of SVZ cells that formed multipotent neurospheres in culture (Fig. 2a), or the self-renewal potential of these cells upon subcloning (Fig. 2b). This is consistent with our failure to detect $p16^{INK4a}$ expression in SVZ cells from young mice *in vivo*, as well as with previous studies^{19,20}. In contrast, $p16^{INK4a}$ deficiency in old mice significantly increased the frequency of SVZ cells that formed

¹Howard Hughes Medical Institute, Department of Internal Medicine, and Center for Stem Cell Biology, University of Michigan, Ann Arbor, Michigan 48109-2216, USA.

²Departments of Medicine and Genetics, The Lineberger Comprehensive Cancer Center, The University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599-7295, USA. †Present address: Laboratorio de Investigaciones Biomedicas, Hospital Universitario Virgen del Rocio, Universidad de Sevilla, E-41013 Seville, Spain.

*These authors contributed equally to this work.

multipotent neurospheres in culture (Fig. 2a; hash, $P < 0.05$ relative to old wild-type mice), as well as the self-renewal potential of these cells (Fig. 2b; hash, $P < 0.05$ relative to old wild-type mice). Consistent with this finding, the rate of proliferation among SVZ cells *in vivo* was also significantly increased by $p16^{INK4a}$ deficiency in old but not young mice (Fig. 2c; hash, $P < 0.05$ relative to old wild-type mice). Nonetheless, the percentage of proliferating cells in old $p16^{INK4a}$ -deficient mice was still significantly less than observed in normal or $p16^{INK4a}$ -deficient young mice (Fig. 2c; asterisk, $P < 0.05$ relative to young mice). Like old wild-type mice, old $p16^{INK4a}$ -deficient mice also exhibited enlarged lateral ventricles due to cortical atrophy (Fig. 1c, data not shown). These observations indicate that $p16^{INK4a}$ deficiency partially rescued the age-related declines in progenitor activity in the SVZ but did not prevent cortical atrophy.

$p16^{INK4a}$ deficiency seemed to rescue completely the age-related decline in cells that can form stem cell colonies in culture (Fig. 2a), while only partially rescuing the overall decline in SVZ proliferation (Fig. 2c). One possibility that would be consistent with previous studies of $p16^{INK4a}$ in neural progenitors^{19–21} is that $p16^{INK4a}$ expression had a greater effect on SVZ stem cells than on downstream progenitors. A number of studies have argued that stem cells can be identified within the SVZ based on their ability to retain the DNA replication label 5-bromodeoxyuridine (BrdU; stem cells divide infrequently and are retained within the SVZ while other progenitors

divide continuously before migrating out of the SVZ)^{22–25}. Therefore, we examined the effects of age and $p16^{INK4a}$ deficiency on the frequency of BrdU label-retaining cells. We administered BrdU for 8 days, followed by a 4-week chase with no BrdU, then killed the mice. $p16^{INK4a}$ deficiency had no effect on the frequency of label-retaining cells in the young adult SVZ (Fig. 2d). The frequency of label-retaining cells declined significantly in old wild-type mice but not in old $p16^{INK4a}$ -deficient mice (Fig. 2d). Thus, the frequencies of label-retaining cells *in vivo* exhibited similar trends as observed for the frequencies of cells that could form multi-lineage colonies in culture. These data suggest that $p16^{INK4a}$ deficiency can largely rescue the age-related decline in the frequency of early progenitors within the SVZ.

SVZ progenitors form neuroblasts throughout life that migrate into the olfactory bulb and differentiate into neurons^{13,22}. To test whether the increase in $p16^{INK4a}$ expression within the SVZ also affects neurogenesis, we examined the effects of age and $p16^{INK4a}$ deficiency on the generation of new neurons in the olfactory bulb. We administered BrdU to mice for 8 days to mark dividing progenitors, followed by a 4-week chase period with no BrdU (during which neurons could migrate and differentiate), then killed the mice to analyse sections through the olfactory bulb by confocal microscopy. As we observed for progenitor function, $p16^{INK4a}$ deficiency had no effect on neurogenesis in young mice, but neurogenesis significantly decreased with age, and $p16^{INK4a}$ deficiency significantly increased the frequency of newly generated olfactory bulb neurons in 15–19-month-old mice (Fig. 3i). Notably, $p16^{INK4a}$ deficiency had no effect on the frequency of non-neuronal cells within the olfactory

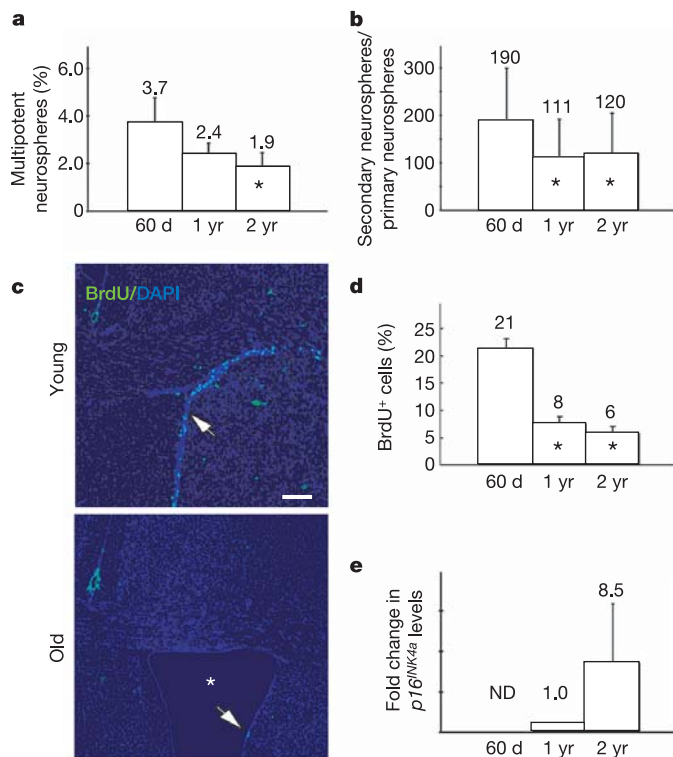


Figure 1 | Neural progenitor function declines with age. **a**, The percentage of SVZ cells that formed multipotent neurospheres in culture declined with age (asterisk, $P < 0.05$ relative to 60-day-old mice; three independent experiments; 5–6 mice per age; error bars for all panels are $\pm$ s.d.). **b**, The self-renewal potential of these primary neurospheres also declined with age (asterisk, $P < 0.05$; three independent experiments; 5–6 mice per age). **c**, **d**, Proliferation in the SVZ (percentage of BrdU⁺ cells after a 2-h pulse) also declined significantly with age (three mice per age; 5–7 sections per mouse). The SVZ thinned in old mice (**c**, arrows), and the lateral ventricle expanded (asterisk) due to cortical atrophy (**c**, lateral ventricle is not visible at this magnification in young mice; scale bar, 200 μ m). **e**, $p16^{INK4a}$ mRNA expression increased with age as detected by quantitative (real-time) PCR in uncultured SVZ cells (three independent samples per age). Note that 1-yr-old samples were set to 1.0 as the reference sample. ND, not detected.

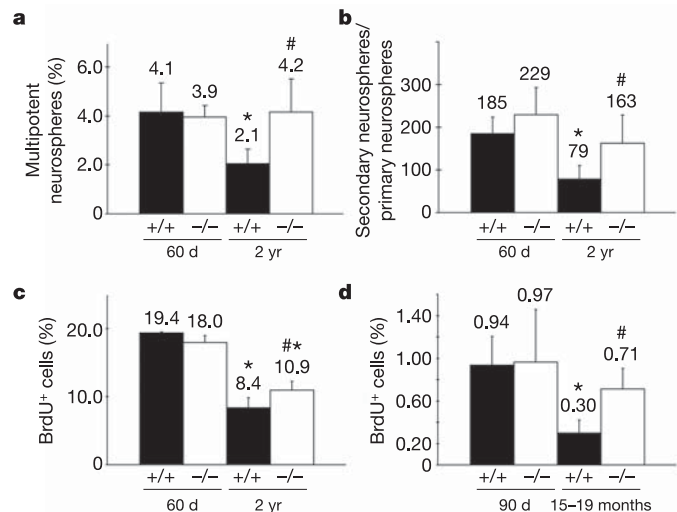


Figure 2 | $p16^{INK4a}$ causes age-related declines in stem and progenitor cell function in the SVZ. **a**, The percentage of SVZ cells that formed multipotent neurospheres in culture significantly declined in 2-yr-old wild-type mice as compared with 60-day-old mice (asterisk, $P < 0.01$) but significantly increased in old mice with $p16^{INK4a}$ deficiency (hash, $P < 0.01$ relative to old wild-type mice). **b**, Self-renewal potential (the number of secondary neurospheres generated per subcloned primary neurosphere) significantly declined in old wild-type mice as compared to young mice (asterisk, $P < 0.05$). $p16^{INK4a}$ deficiency significantly increased the self-renewal of neurospheres from old but not young mice (hash, $P < 0.05$ relative to old wild-type mice). **c**, The percentage of SVZ cells that incorporated a 2-h pulse of BrdU significantly declined in old as compared to young mice (asterisk, $P < 0.01$), but significantly increased in old mice with $p16^{INK4a}$ deficiency (hash, $P < 0.01$ relative to old wild-type mice). **d**, The frequency of BrdU label-retaining cells in the SVZ significantly declined in old wild-type mice as compared with young wild-type mice (asterisk, $P < 0.05$), but significantly increased in old mice with $p16^{INK4a}$ deficiency (hash, $P < 0.05$ relative to old wild-type mice). All values are mean $\pm$ s.d. for at least three independent experiments. All mice were histologically negative for intracranial neoplasms.

bulb (Fig. 3j). This may at least partially reflect our previous observation that changes in $p16^{INK4a}$ expression do not appear to affect glia-restricted progenitors²⁰. $p16^{INK4a}$ deficiency thus partially rescued the age-related decline in neurogenesis in the olfactory bulb in addition to rescuing partially the decline in progenitor function in the SVZ.

To test whether these effects of $p16^{INK4a}$ occurred throughout the CNS, we also examined the effect of $p16^{INK4a}$ deficiency on progenitor activity and neurogenesis in the dentate gyrus²⁶. The rates of progenitor proliferation and neurogenesis in the dentate gyrus decline markedly with age¹². To test whether this is affected by $p16^{INK4a}$, we administered BrdU for 8 days to mark proliferation in the subgranular layer, followed by a 4-week chase period without BrdU to examine neurogenesis in the granular layer. $p16^{INK4a}$ deficiency did not significantly affect the rate of proliferation among progenitors in the subgranular layer, or the frequency of BrdU⁺NeuN⁺ newly generated neurons or BrdU⁺NeuN⁻ non-neuronal cells in the granular layer of the dentate gyrus (Supplementary Fig. 1). Thus, although $p16^{INK4a}$ deficiency consistently increased all measures of progenitor activity and neurogenesis in the ageing subventricular zone/olfactory bulb, it did not detectably affect proliferation or neurogenesis in the dentate gyrus.

We also examined the effect of age and $p16^{INK4a}$ deficiency on the neural crest stem cells that persist throughout adult life in the enteric nervous system (in the gut wall)^{19,20,27}. The frequency of these neural crest stem cells declined with age, and $p16^{INK4a}$ expression increased with age in p75⁺ (neurotrophin-receptor expressing) gut cells enriched for neural crest stem cells (Supplementary Fig. 2). However, $p16^{INK4a}$ deficiency had no effect on neural crest stem cell frequency in young or old mice. These results indicate that although the age-related increase in $p16^{INK4a}$ expression causes a decline in SVZ progenitor function and neurogenesis, other mechanisms are more important in the ageing of stem and progenitor cells in the hippocampus and peripheral nervous system.

The mechanisms that account for the increase in $p16^{INK4a}$ expression with age remain unclear. In principle, $p16^{INK4a}$ expression could be developmentally programmed to increase with age to

counter the increasing incidence of cancer that is observed in the ageing nervous system. Alternatively, $p16^{INK4a}$ expression may reflect the induction of senescence in ageing cells in response to damage that accumulates from oxidative metabolism or other stresses. Although Bmi1 is important for $p16^{INK4a}$ repression^{19,20,28}, we did not detect any change in Bmi1 expression with age at the RNA or protein levels in SVZ cells (Supplementary Fig. 3). Nonetheless, subtle reductions in Bmi1 expression or activity cannot be excluded and could be functionally important, as even the twofold reduction in Bmi1 expression in $Bmi1^{-/+}$ mice leads to increased $p16^{INK4a}$ expression (data not shown). Additional work will be required to resolve the potentially multifactorial mechanisms that regulate age-related changes in $p16^{INK4a}$ expression.

The simplest interpretation of our data is that $p16^{INK4a}$ acts cell autonomously within neural stem cells, because $p16^{INK4a}$ deficiency increased the self-renewal of single neural stem cells in culture. However, we cannot exclude the possibility of non-cell-autonomous effects by $p16^{INK4a}$ *in vivo*. In addition to investigating this issue further, it will also be of interest to determine whether circulating humoral factors¹¹ influence $p16^{INK4a}$ expression during ageing.

One study found that moderately increased expression of $p16^{INK4a}$ and $p19^{ARF}$ in transgenic mice does not detectably accelerate gross measures of ageing or change lifespan, despite reducing cancer incidence²⁹. However, that study did not examine neural progenitor function or neurogenesis. We also examined $p16^{INK4a}$ bacterial artificial chromosome (BAC) transgenic mice that exhibited a moderate increase in $p16^{INK4a}$ expression and found no effect of $p16^{INK4a}$ overexpression on stem cell frequency or self-renewal potential, although old transgenic mice did exhibit a modest reduction in SVZ proliferation (data not shown). It seems that the levels of $p16^{INK4a}$ that are required to deplete stem cells are qualitatively higher than the levels that are required to inhibit carcinogenesis. Together, the data suggest that although physiological increases in $p16^{INK4a}$ expression during ageing reduce neural progenitor function and neurogenesis in the SVZ/olfactory bulb, modest overexpression of $p16^{INK4a}$ does not substantially amplify these

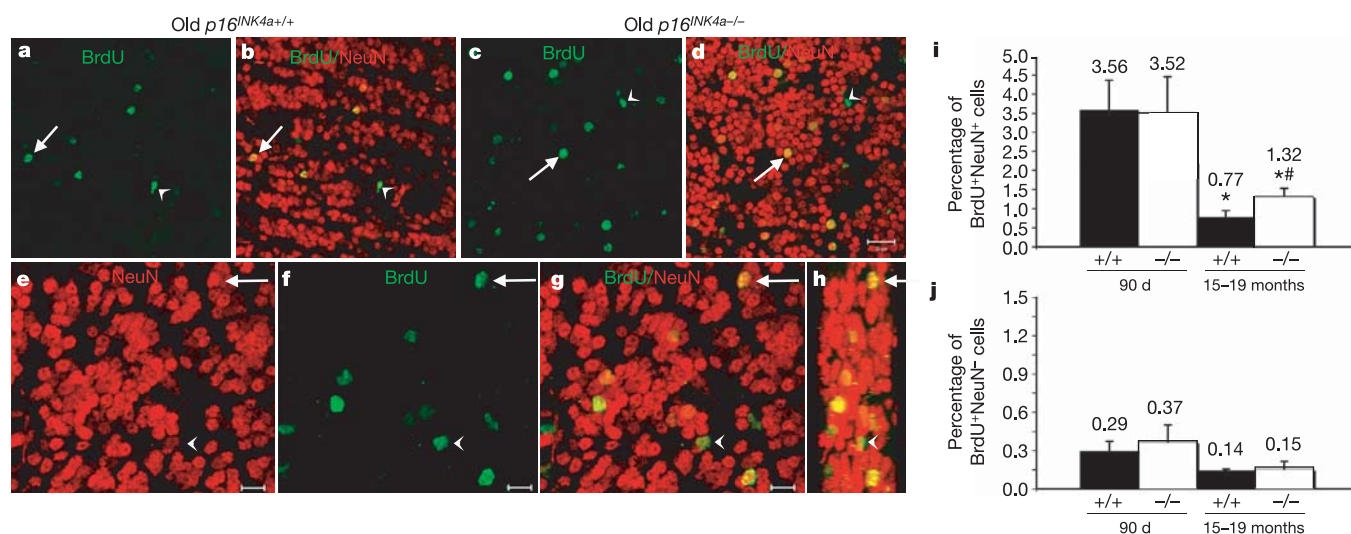


Figure 3 | $p16^{INK4a}$ causes age-related declines in olfactory bulb neurogenesis. **a–d**, Low-magnification images (scale bar, 20 μ m) from sagittal sections of the olfactory bulb of old wild-type (**a**, **b**; same field of view) and $p16^{INK4a}$ -deficient (**c**, **d**; same field of view) mice. Arrows point to new neurons in the granular layer (BrdU⁺NeuN⁺) whereas arrowheads point to new non-neuronal cells (BrdU⁺NeuN⁻). **e–h**, Higher magnification images (scale bar, 10 μ m) from one field of view from an old $p16^{INK4a}$ -deficient mouse. Arrow indicates BrdU⁺NeuN⁺ neuron; arrowhead indicates BrdU⁺NeuN⁻ non-neuronal cell. Panel **h** is a three-dimensional, reconstructed side view (80° turn in the z-axis) of panel **g**. **i**, Neurogenesis

significantly (asterisk, $P < 0.05$) declined with age (BrdU⁺NeuN⁺ neurons as a percentage of all NeuN⁺ neurons). $p16^{INK4a}$ deficiency did not affect the level of neurogenesis in young mice, but significantly (hash, $P = 0.02$ relative to old wild-type mice) increased neurogenesis in old mice. **j**, The frequency of BrdU⁺NeuN⁻ non-neuronal cells was not significantly affected by $p16^{INK4a}$ deficiency (also as a percentage of NeuN⁺ neurons). The same trends were observed when the counts were expressed per unit area (not shown). Values are mean $\pm$ s.d. from 25 to 30 fields of view per mouse, three mice per treatment.

effects or accelerate gross measures of ageing.

Although physiological $p16^{INK4a}$ expression reduces stem/progenitor cell function and neurogenesis with age, how this relates to overall ageing or lifespan remains unclear. It is difficult to examine the effect of $p16^{INK4a}$ deficiency on mouse lifespan because $p16^{INK4a}$ deficiency increases cancer incidence³⁰ in addition to affecting age-related changes in stem/progenitor cell function. Declining stem/progenitor cell function may be a major cause of the decline in regenerative capacity and the increase in degenerative disease that is observed in ageing tissues. On the other hand, increases in the death or dysfunction of mature cells in ageing tissues also contribute to age-related morbidity. It is similarly unknown whether physiological differences in the rate at which stem/progenitor cell activity declines with age has a detectable impact on longevity. The fact that $p16^{INK4a}$ did not affect progenitors in certain regions of the nervous system suggests that $p16^{INK4a}$ is not likely to promote generically the ageing of all cells. Indeed, it is likely that there will be important differences between tissues, and perhaps between progenitor cells and differentiated cells, in terms of the genes that regulate the ageing process. Although these questions remain unanswered, it may be possible to gain important new insights into the ageing process at a cellular level by studying individual cell types that have known physiological functions *in vivo*, such as haematopoietic or neural stem cells.

Our data suggest that stem cell function is regulated by a balance between proto-oncogenes, like *Bmi1* (that promote stem cell maintenance and regenerative capacity but can also contribute to neoplastic proliferation), and tumour suppressors, like $p16^{INK4a}$ (that reduce regenerative capacity and promote ageing but also reduce cancer incidence). This balance changes with age and is influenced by other proto-oncogenes and tumour suppressors that also regulate $p16^{INK4a}$ expression. The networks of proto-oncogenes and tumour suppressors that regulate stem cell self-renewal, cancer cell proliferation and stem cell ageing may have evolved to balance the need for regenerative capacity while guarding against the increasing risk of neoplasms with age. $p16^{INK4a}$ thus reduces cancer incidence^{5,30} but also contributes to ageing by reducing progenitor function and neurogenesis in at least certain regions of the nervous system.

METHODS

Mice. $p16^{INK4a+/-}$ mice were backcrossed at least six times onto a C57BL background. $p16^{INK4a}$ genotyping was performed by PCR as described³⁰.

Isolation of CNS progenitors. Adult SVZ was obtained by microdissecting the lateral walls of the lateral ventricles, then dissociating for 20 min at 37°C in 0.025% trypsin/0.5 mM EDTA (Calbiochem) plus 0.001% DNaseI (Roche). After quenching the enzymatic dissociation with staining medium (L15 medium containing 1 mg ml⁻¹ BSA (Sigma A-3912), 10 mM HEPES (pH 7.4) and 1% penicillin/streptomycin (BioWhittaker)) containing 0.014% soybean trypsin inhibitor (Sigma) and 0.001% DNaseI, the cells were washed and re-suspended in staining medium, triturated, filtered through nylon screen (45 µm, Sefar America), counted by haemocytometer, and plated.

Cell culture and self-renewal assay. Cells were plated at clonal density (1.3 cells per µl) on ultra-low-binding plates (Corning) to grow neurospheres. Culture medium was based on a 5:3 mixture of DMEM-low glucose as described previously¹⁹: neurobasal medium (Gibco) supplemented with 20 ng ml⁻¹ human bFGF (R&D Systems), 20 ng ml⁻¹ EGF (R&D Systems), 1% N2 supplement (Gibco), 2% B27 supplement (Gibco), 50 µM 2-mercaptoethanol, 1% penicillin/streptomycin (BioWhittaker), and 10% chick embryo extract. Cultures were maintained at 37°C in 6% CO₂/balance air. To measure self-renewal, individual neurospheres were dissociated by trituration then replated at clonal density as above. Secondary neurospheres were counted 5–10 days later to determine the number of secondary neurospheres formed per primary neurosphere. CNS neurospheres were tested for multipotency by replating one neurosphere per well into 48-well plates and then culturing adherently for 3–5 days before triple staining for oligodendrocytes (O4), neurons (TuJ1) and astrocytes (GFAP). See Supplementary Methods for details.

BrdU incorporation/proliferation assays. To quantify SVZ proliferation, mice were injected intraperitoneally with 50 mg kg⁻¹ of BrdU (Sigma), and killed 2 h after BrdU injection. To quantify proliferation in the dentate gyrus, mice received a single injection of 50 mg kg⁻¹ BrdU, followed by 1 mg ml⁻¹ BrdU in their drinking water for 8 days before being killed and analysed. To quantify

neurogenesis in the dentate gyrus and the olfactory bulb and BrdU retention in the SVZ, mice were treated with BrdU for 8 days. However, the animals were then taken off of BrdU for 4 weeks before the animals were killed. The brains were dissected, fixed in 4% paraformaldehyde overnight, then cryo-protected in 15% sucrose, embedded in 7.5% gelatin/15% sucrose, and flash frozen. Twelve-micrometre sections were cut on a Leica cryostat.

For detection of BrdU in the tissue sections, DNA was denatured in 2 M HCl for 30 min at room temperature and neutralized with 0.1 M sodium borate. Sections were pre-blocked for 1 h at room temperature in goat serum solution (PBS containing 5% goat serum, 1% BSA and 0.3% Triton X-100 (Sigma)). Primary rat anti-BrdU (1:500, Accurate Chemical) diluted in goat serum solution was incubated overnight at 4°C, followed by fluorescein isothiocyanate (FITC)-conjugated anti-rat Fc fragment (Jackson Labs) for 3–4 h at room temperature. Slides were counter stained in 2.5 µg ml⁻¹ DAPI for 10 min at room temperature, then mounted using ProLong antifade solution (Molecular Probes). Numbers of BrdU-labelled cells were divided by total DAPI⁺ or NeuN⁺ nuclei.

To ensure that BrdU incorporation results were not skewed by dividing endothelial or haematopoietic cells, we double-labelled SVZ sections with antibodies against BrdU and either anti-CD45 (to identify blood cells) or anti-VE-cadherin (to identify endothelial cells). We did not detect any BrdU⁺ haematopoietic or endothelial cells.

Western blots and quantitative RT-PCR were performed as described in Supplementary Information.

Statistical analysis. Experiments that involved multiple treatments were initially analysed by analysis of variance. Statistically significant results were followed up with Student's *t*-tests.

Confocal analysis of neurogenesis in the olfactory bulb. A Zeiss LSM 510 confocal laser-scanning microscope was used to obtain 25–30 random fields of view throughout all regions of one entire olfactory bulb of each mouse with a ×40 or ×63 objective lens. For each image, 1-µm-thick optical sections were scanned with three different lasers through a 12-µm sagittal tissue section creating a Z-series stack with three distinct channels of fluorescence. An ultraviolet enterprise laser was used to detect the DAPI signal labelling all nuclei. An argon laser detected FITC (BrdU), and a HeNe laser detected Cy3 (NeuN). Channels were merged together to determine whether DAPI, BrdU and NeuN signals co-labelled at every 1-µm slice of the Z-series. Three-dimensional projections were made using LSM 510 software.

Received 10 April; accepted 25 July 2006.

Published online 6 September 2006.

- Chien, K. R. & Karsenty, G. Longevity and lineages: toward the integrative biology of degenerative diseases in heart, muscle, and bone. *Cell* **120**, 533–544 (2005).
- Geiger, H. & Van Zant, G. The aging of lympho-hematopoietic stem cells. *Nature Immunol.* **3**, 329–333 (2002).
- Lombard, D. B. *et al.* DNA repair, genome stability, and aging. *Cell* **120**, 497–512 (2005).
- Campisi, J. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. *Cell* **120**, 513–522 (2005).
- Lowe, S. W. & Sherr, C. J. Tumor suppression by *Ink4a-Arf*: progress and puzzles. *Curr. Opin. Genet. Dev.* **13**, 77–83 (2003).
- Maslov, A. Y., Barone, T. A., Plunkett, R. J. & Pruitt, S. C. Neural stem cell detection, characterization, and age-related changes in the subventricular zone of mice. *J. Neurosci.* **24**, 1726–1733 (2004).
- Morrison, S. J., Wandycz, A. M., Akashi, K., Globerson, A. & Weissman, I. L. The aging of hematopoietic stem cells. *Nature Med.* **2**, 1011–1016 (1996).
- Chen, J., Astle, C. M. & Harrison, D. E. Genetic regulation of primitive hematopoietic stem cell senescence. *Exp. Hematol.* **28**, 442–450 (2000).
- de Haan, G., Nijhof, W. & Van Zant, G. Mouse strain-dependent changes in frequency and proliferation of hematopoietic stem cells during aging: correlation between lifespan and cycling activity. *Blood* **89**, 1543–1550 (1997).
- Conboy, I. M., Conboy, M. J., Smythe, G. M. & Rando, T. A. Notch-mediated restoration of regenerative potential to aged muscle. *Science* **302**, 1575–1577 (2003).
- Conboy, I. M. *et al.* Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* **433**, 760–764 (2005).
- Kuhn, H. G., Dickinson-Anson, H. & Gage, F. H. Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J. Neurosci.* **16**, 2027–2033 (1996).
- Enwere, E. *et al.* Aging results in reduced epidermal growth factor receptor signaling, diminished olfactory neurogenesis, and deficits in fine olfactory discrimination. *J. Neurosci.* **24**, 8354–8365 (2004).
- Nielsen, G. P. *et al.* Immunohistochemical survey of p16INK4A expression in normal human adult and infant tissues. *Lab. Invest.* **79**, 1137–1143 (1999).

15. Krishnamurthy, J. *et al.* *Ink4a/Arf* expression is a biomarker of aging. *J. Clin. Invest.* **114**, 1299–1307 (2004).
16. Zindy, F., Quelle, D. E., Roussel, M. F. & Sherr, C. J. Expression of the p16^{INK4a} tumor suppressor versus other INK4 family members during mouse development and aging. *Oncogene* **15**, 203–211 (1997).
17. Sharpless, N. E., Ramsey, M. R., Balasubramanian, P., Castrillon, D. H. & DePinho, R. A. The differential impact of p16^{INK4a} or p19^{ARF} deficiency on cell growth and tumorigenesis. *Oncogene* **23**, 379–385 (2004).
18. Michaloglou, C. *et al.* BRAFE600-associated senescence-like cell cycle arrest of human naevi. *Nature* **436**, 720–724 (2005).
19. Molofsky, A. V. *et al.* Bmi-1 promotes neural stem cell self-renewal and neural development but not mouse growth and survival by repressing the p16^{INK4a} and p19^{ARF} senescence pathways. *Genes Dev.* **19**, 1432–1437 (2005).
20. Molofsky, A. V. *et al.* Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation. *Nature* **425**, 962–967 (2003).
21. Bruggeman, S. W. M. *et al.* *Ink4a* and *Arf* differentially affect cell proliferation and neural stem cell self-renewal in *Bmi1*-deficient mice. *Genes Dev.* **19**, 1438–1443 (2005).
22. Doetsch, F., Caille, I., Lim, D. A., Garcia-Verdugo, J. M. & Alvarez-Buylla, A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* **97**, 703–716 (1999).
23. Capela, A. & Temple, S. LeX/ssea-1 is expressed by adult mouse CNS stem cells, identifying them as nonependymal. *Neuron* **35**, 865–875 (2002).
24. Chiasson, B. J., Tropepe, V., Morshead, C. M. & Kooy, D. v. d. Adult mammalian forebrain ependymal and subependymal cells demonstrate proliferative potential, but only subependymal cells have neural stem cell characteristics. *J. Neurosci.* **19**, 4462–4471 (1999).
25. Johansson, C. B. *et al.* Identification of a neural stem cell in the adult mammalian central nervous system. *Cell* **96**, 25–34 (1999).
26. van Praag, H. *et al.* Functional neurogenesis in the adult hippocampus. *Nature* **415**, 1030–1034 (2002).
27. Kruger, G. M. *et al.* Neural crest stem cells persist in the adult gut but undergo changes in self-renewal, neuronal subtype potential, and factor responsiveness. *Neuron* **35**, 657–669 (2002).
28. Jacobs, J. J. L., Kieboom, K., Marino, S., DePinho, R. A. & Lohuizen, M. v. The oncogene and polycomb-group gene *bmi-1* regulates cell proliferation and senescence through the *ink4a* locus. *Nature* **397**, 164–168 (1999).
29. Matheu, A. *et al.* Increased gene dosage of *Ink4a/Arf* results in cancer resistance and normal aging. *Genes Dev.* **18**, 2736–2746 (2004).
30. Sharpless, N. E. *et al.* Loss of p16^{INK4a} with retention of p19^{ARF} predisposes mice to tumorigenesis. *Nature* **413**, 86–91 (2001).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements This work was supported by the National Institute on Aging (grants to S.J.M. and N.E.S.) and the National Institute of Neurological Disorders and Stroke (to S.J.M.). S.J.M. is an Investigator of the Howard Hughes Medical Institute. N.E.S. is supported by the Sidney Kimmel Foundation for Cancer Research, the Paul Beeson Physician Scholars program, and the Ellison Medical Foundation. A.V.M. and N.M.J. were supported by National Research Service Awards from the National Institutes of Health. We thank K. Yeager for tissue sectioning and C. Mountford for mouse colony management.

Author Contributions A.V.M. studied the effect of age on forebrain progenitors, p16^{INK4a} expression and function during ageing in the subventricular zone (Figs 1 and 2), and Bmi1 expression during ageing (Supplementary Fig. 3). S.G.S. contributed to studies of p16^{INK4a} expression during ageing, and the effect of p16^{INK4a} on proliferation and neurogenesis in the subventricular zone and hippocampus (Figs 2 and 3 and Supplementary Fig. 1). N.M.J. studied neurogenesis in the olfactory bulb and hippocampus (Fig. 3 and Supplementary Fig. 1). S.H. and R.P. examined p16^{INK4a} expression in the ageing enteric nervous system and its effect on neural crest stem cells (Supplementary Fig. 2). J.K. and N.E.S. provided ageing p16^{INK4a}-deficient and control mice for some of the experiments and discussed results throughout the project. S.J.M. helped to design and interpret experiments and wrote the manuscript with help from A.V.M., S.G.S. and N.M.J.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to S.J.M. (seanjm@umich.edu).

p16^{INK4a} induces an age-dependent decline in islet regenerative potential

Janakiraman Krishnamurthy¹, Matthew R. Ramsey¹, Keith L. Ligon², Chad Torrice¹, Angela Koh³, Susan Bonner-Weir³ & Norman E. Sharpless¹

The p16^{INK4a} tumour suppressor accumulates in many tissues as a function of advancing age^{1–3}. p16^{INK4a} is an effector of senescence^{4,5} and a potent inhibitor of the proliferative kinase Cdk4 (ref. 6), which is essential for pancreatic β -cell proliferation in adult mammals^{7,8}. Here we show that p16^{INK4a} constrains islet proliferation and regeneration in an age-dependent manner. Expression of the p16^{INK4a} transcript is enriched in purified islets compared with the exocrine pancreas, and islet-specific expression of p16^{INK4a}, but not other cyclin-dependent kinase inhibitors, increases markedly with ageing. To determine the physiological significance of p16^{INK4a} accumulation on islet function, we assessed the impact of p16^{INK4a} deficiency and overexpression with increasing age and in the regenerative response after exposure to a specific β -cell toxin. Transgenic mice that overexpress p16^{INK4a} to a degree seen with ageing demonstrated decreased islet proliferation. Similarly, islet proliferation was unaffected by p16^{INK4a} deficiency in young mice, but was relatively increased in p16^{INK4a}-deficient old mice. Survival after toxin-mediated ablation of β -cells, which requires islet proliferation, declined with advancing age; however, mice lacking p16^{INK4a} demonstrated enhanced islet proliferation and survival after β -cell ablation. These genetic data support the view that an age-induced increase of p16^{INK4a} expression limits the regenerative capacity of β -cells with ageing.

Mammalian homeostasis depends on the function of reservoirs of proliferating cells necessary for tissue regeneration, and therefore regulators of proliferation are thought to be involved in ageing^{4,5}. In particular, p16^{INK4a}, a product of the *INK4a/ARF* (*Cdkn2a*) locus, has been postulated to contribute to ageing. Although its expression increases with age in most tissues^{1–3}, we chose to study its role in the pancreatic islet for several reasons. Expression of p16^{INK4a} sharply increases with ageing in the endocrine pancreas of rodents and humans^{2,3}. Cultured human islets undergo telomere-independent senescence in the setting of p16^{INK4a} induction⁹, suggesting that p16^{INK4a} can be a principal mediator of proliferation in this cell type. Mice lacking *Cdk4* or cyclin D1/2, which activate Cdk4, are born with normal islet mass, but develop diabetes in the setting of reduced proliferation and islet mass as young adults^{7,8,10}. Mice with increased islet Cdk4 activity, because of p18^{INK4c} deficiency or activating mutations of Cdk4 (refs 11–13), show an increase in islet mass. Therefore, the islet seems to be an ideal compartment for determining the consequences of p16^{INK4a}, as expression increases with ageing in this tissue, and the principal biochemical target of p16^{INK4a} has a clear role in β -cell proliferation.

To define the compartmental and age-related expression of p16^{INK4a}, we isolated islets from total pancreas as described¹⁴. Total

RNA was harvested from islets and exocrine pancreas from 12–14-week-old and 64–80-week-old mice, and the expression of indicated transcripts determined by quantitative TaqMan analysis (Fig. 1a, b and Supplementary Fig. 1). Using this approach, we detected p16^{INK4a} expression in the islets of young animals, which increased by 14-fold (mean value) with 52–66 weeks of additional ageing. Expression of p16^{INK4a} was enriched in purified islets, with >30-fold higher expression than in the exocrine pancreas. As is the case in most rodent tissues^{1,2}, expression of *Arf*, the other *Ink4a/Arf* transcript, mirrored p16^{INK4a}, with a similar degree of age-induced increase and islet enrichment. However, the messenger RNA expression of other cyclin-dependent kinase inhibitors in this compartment changed only modestly or not at all with ageing (Fig. 1b and Supplementary

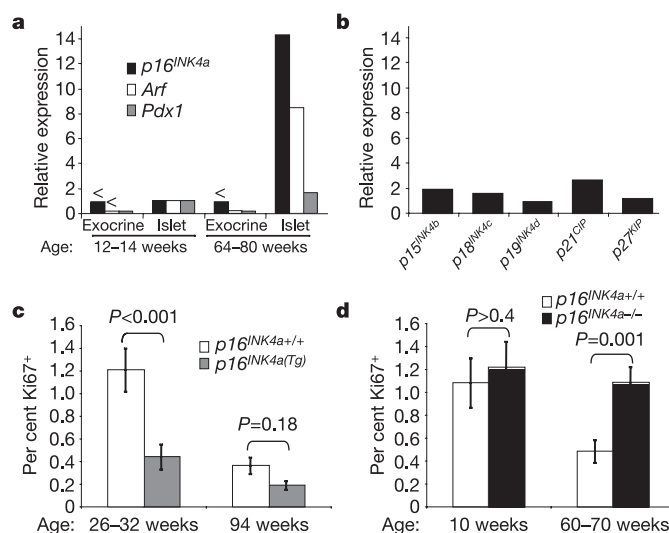


Figure 1 | Islet gene expression and proliferation in young versus old mice. **a**, Expression of p16^{INK4a}, Arf and Pdx1 as determined by TaqMan on islets and exocrine pancreas from mice of indicated ages. Relative expression is shown normalized to islet expression in young mice. ‘<’ indicates expression beneath the lower limit of detection. **b**, Relative mRNA expression of the indicated genes in islets with ageing. Values are reported as mean ratio of expression in old (64–80 weeks) versus young (12–14 weeks) mice. **c**, Per cent Ki67-positive cells of pancreatic islets from p16^{INK4a} wild-type and overexpressing BAC transgenic mice of indicated ages. Error bars indicate $\pm$ s.e.m.; P-values were determined by Mann–Whitney U-test. **d**, Per cent Ki67-positive cells of pancreatic islets from p16^{INK4a}^{+/+} and p16^{INK4a}^{-/-} mice of indicated ages. Error bars indicate $\pm$ s.e.m.; P-values were determined by Mann–Whitney U-test.

¹Departments of Medicine and Genetics, The Lineberger Comprehensive Cancer Center, The University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599, USA. ²Department of Pathology, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts 02115, USA. ³Joslin Diabetes Center and Harvard Medical School, Boston, Massachusetts 02115, USA.

Fig. 1). Notably, both products of the *Ink4a/Arf* locus were more enriched in the islet than *Pdx1* (Fig. 1a), a transcription factor mainly restricted to β -cells in adults¹⁵. These data demonstrate that islet expression of the *Ink4a/Arf* locus is comparable to that seen in other highly expressing tissues such as spleen or lung² and sharply increases with ageing. Moreover, although other cell cycle inhibitors such as *p18^{INK4c}* and *p27^{KIP1}* have a more important role in determining β -cell proliferation and number during development and young adulthood^{13,16}, the marked transcriptional increase with ageing seems to be a unique property among cell cycle inhibitors of the products of the *Ink4a/Arf* locus.

We did not detect a difference in either the total number of cells or the number of proliferating cells per islet in young *p16^{INK4a}*-deficient animals compared to controls. To test whether an increase in *p16^{INK4a}* level comparable to that seen with ageing (Fig. 1a) would regulate islet proliferation, we generated *p16^{INK4a}* bacterial artificial chromosome (BAC) transgenic mice harbouring a single extra copy of the *p16^{INK4a}* gene (Supplementary Fig. 2). We identified a line (*p16^{INK4a}(Tg)*) that demonstrated a mean fivefold increase in expression of *p16^{INK4a}* mRNA and protein across a panel of tissues including the pancreas (Supplementary Figs 2 and 3). In young transgenic mice, *p16^{INK4a}* expression was comparable to that in ~60-week-old wild-type mice. Transgenic animals demonstrated normal size, fertility and glucose metabolism (not shown). A reduction in proliferation was noted in islets from *p16^{INK4a}(Tg)* mice compared to littermate controls at all ages, but the effect was greatest in young mice (Fig. 1c). This observation suggests that the level of *p16^{INK4a}* expression detected in ~60-week-old animals inhibits islet proliferation.

To determine the effects of endogenous *p16^{INK4a}* with ageing, islet proliferation in young and old *p16^{INK4a}/+* and *p16^{INK4a}/-* mice was analysed (Fig. 1d). No evidence of pancreatic neoplasia was

noted in *p16^{INK4a}*-deficient animals, and germline deficiency of *p16^{INK4a}* did not influence weight in mice less than a year of age (Supplementary Fig. 4). Islet proliferation measured by calculating the Ki67⁺ proliferation index significantly decreased with ageing in *p16^{INK4a}/+* mice (from 1.21% of islet cells in 20-week-old mice to 0.36% of cells in 94-week-old mice; $P < 0.001$ for trend, Fig. 1c, d). Although germline *p16^{INK4a}* inactivation did not affect islet proliferation in young mice, the age-induced decrease in proliferation was largely rescued by *p16^{INK4a}* deficiency (Fig. 1d). We believe that complete rescue of islet proliferation is not noted in *p16^{INK4a}/-* mice because these animals demonstrate unperturbed *Arf* expression, which also increases with ageing in this tissue (Fig. 1a and not shown), as well as the possible existence of *Ink4a/Arf*-independent mechanisms of ageing. In aggregate, the results from the BAC transgenic and germline knockout strains demonstrate that with ageing, *p16^{INK4a}* expression becomes a significant regulator of islet proliferation.

The tumour-prone nature^{17,18} of *p16^{INK4a}*-deficient animals limited our ability to determine the functional significance of this inhibition of proliferation imposed by *p16^{INK4a}* with ageing. For example, assays of glucose tolerance even in mice of intermediate age (40–52 weeks old) were confounded by initially undetected tumours in the *p16^{INK4a}/-* cohort (Supplementary Fig. 4). Thus, to study the effect of *p16^{INK4a}* expression on islet function in young adult animals, we adopted a model of islet regeneration after treatment with streptozotocin (STZ). STZ is a specific β -cell toxin that induces necrosis and diabetes when given as a single dose to adult mice, and moderate β -cell regeneration after STZ treatment is well described^{19–21}. After determining a STZ dose (150 mg kg⁻¹) that led to death from diabetes within 100 days of injection in ~75% of 10-week-old C57BL/6 mice, we harvested and analysed pancreata at indicated times after STZ injection. Islet proliferation was signifi-

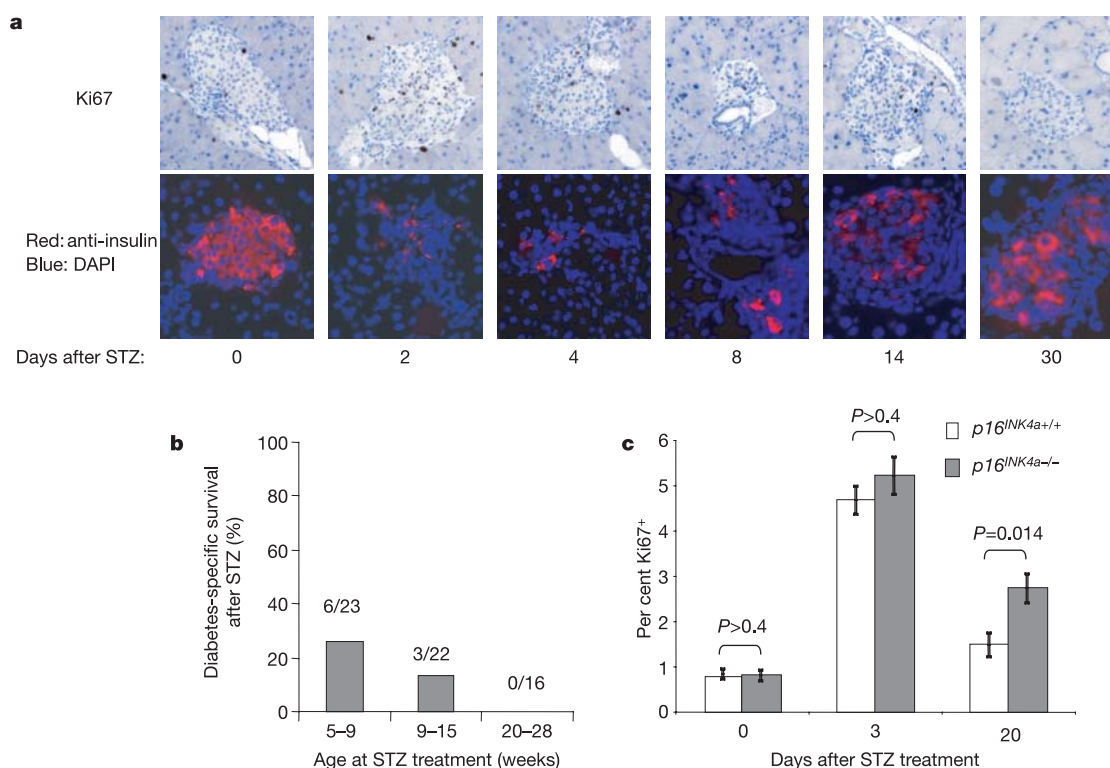


Figure 2 | Islet regeneration after STZ treatment. **a**, Ki67 immunohistochemistry and anti-insulin/DAPI immunofluorescence taken at indicated times after STZ treatment. Original magnification, $\times 200$. **b**, Survival of wild-type C57BL/6 mice after STZ treatment by age on the day of STZ injection. Acute STZ deaths (within 5 days of treatment) are censored from

the analysis. Total numbers of long-term (>100 days after STZ) survivors over total number of mice treated per age are indicated. **c**, Per cent Ki67-positive cells of pancreatic islets from 33-week-old *p16^{INK4a}/+* and *p16^{INK4a}/-* mice at indicated time points after STZ treatment. Error bars indicate $\pm$ s.e.m.; P -values were determined by Mann–Whitney U -test.

cantly increased 2 days after STZ treatment, and remained elevated for 2 weeks after STZ treatment (Supplementary Fig. 5). Double-labelling with Ki67 and insulin demonstrated that >80% of proliferating cells in the islets of untreated mice or after STZ treatment were β -cells, suggesting that islet proliferation and β -cell proliferation strongly correlate in this system.

Islets were depleted of β -cells at 2 and 4 days after STZ treatment, although median cell number per islet did not reach its lowest point until 8 days after STZ treatment (Fig. 2a and Supplementary Fig. 5). At this time point, small, insulin-positive islets associated with ducts were evident, with increasing islet size and insulin expression 14 and 30 days after STZ treatment. Mice surviving more than 30 days after STZ treatment demonstrated a partial recovery of islet size relative to pre-treatment levels. All mice surviving 100 days after STZ treatment showed recovery of β -cell function as demonstrated by resolution of hyperglycaemia and weight gain in excess of pre-treatment weight

(Fig. 3a–c). Notably, islet regeneration after STZ treatment was age dependent (Fig. 2b). Greater than 25% of 5–9-week-old wild-type mice survived more than 100 days after STZ treatment, whereas no wild-type animal older than 20 weeks demonstrated survival after STZ ($P = 0.026$). This does not reflect increased sensitivity to STZ in older cohorts, as old adult mice have been shown to be less sensitive to the β -cell-damaging effects of STZ²⁰. These data suggest that prolonged survival after a single diabetes-causing dose of STZ requires regeneration of significant numbers of functional β -cells, an ability that declines with ageing.

To determine whether $p16^{\text{INK4a}}$ expression contributes to the age-dependent decline in β -cell regeneration, we performed STZ challenge in littermate cohorts of animals of indicated ages. Mice were treated with STZ, and followed serially for islet proliferation (Fig. 2c), weight (Fig. 3a), serum glucose levels (Fig. 3b, c) and diabetes-specific survival (Fig. 4a–c). In 33-week-old STZ-treated mice, islet proliferation was similar in $p16^{\text{INK4a}+/+}$ and $p16^{\text{INK4a}-/-}$ mice before and 3 days after treatment. A significant increase in proliferation, however, was noted in $p16^{\text{INK4a}-/-}$ mice at 20 days after STZ treatment (Fig. 2c). This effect, only at the later time point after islet ablation, probably reflects a delayed (>2 weeks) induction of $p16^{\text{INK4a}}$ expression after a DNA damaging stimulus, as has been described in other systems^{22–25}. These data indicate that $p16^{\text{INK4a}}$ expression limits islet proliferation after β -cell depletion by STZ.

Survival was measured to determine whether differences in islet proliferation after STZ treatment translated into benefit for $p16^{\text{INK4a}}$ -deficient animals. No effect of $p16^{\text{INK4a}}$ deficiency on STZ toxicity was noted in 5–9-week-old mice (Fig. 4a), but

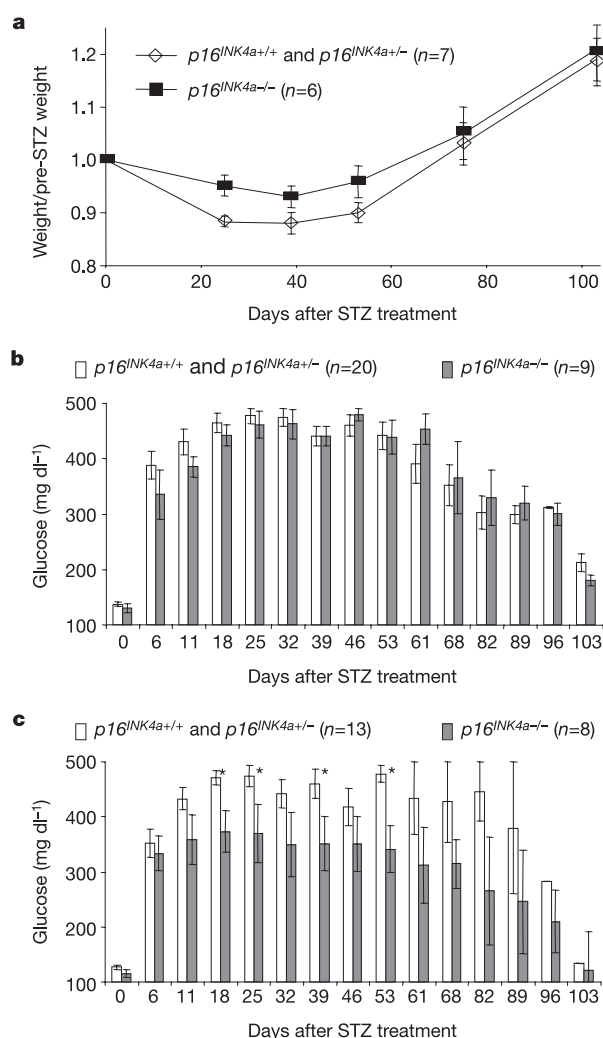


Figure 3 | Metabolic effects of $p16^{\text{INK4a}}$ expression after STZ treatment. **a**, Animal weight after STZ treatment normalized to pre-treatment weight of long-term (>100 days) surviving mice (5–28 weeks of age) by indicated genotypes. All long-term surviving animals showed improved hyperglycaemia and began to regain weight within 55 days after STZ treatment, regardless of genotype. Error bars indicate $\pm$ s.e.m. **b**, **c**, Fasting serum glucose levels after STZ treatment. No difference in fasting serum glucose or survival was seen in mice less than 9 weeks of age after STZ treatment (**b**), but hyperglycaemia was more marked and persistent after STZ treatment in mice greater than 9 weeks of age (9–15 weeks) (**c**). Results are shown $\pm$ s.e.m.; asterisks indicate $P < 0.05$ on the indicated days as determined by two-sided t -test.

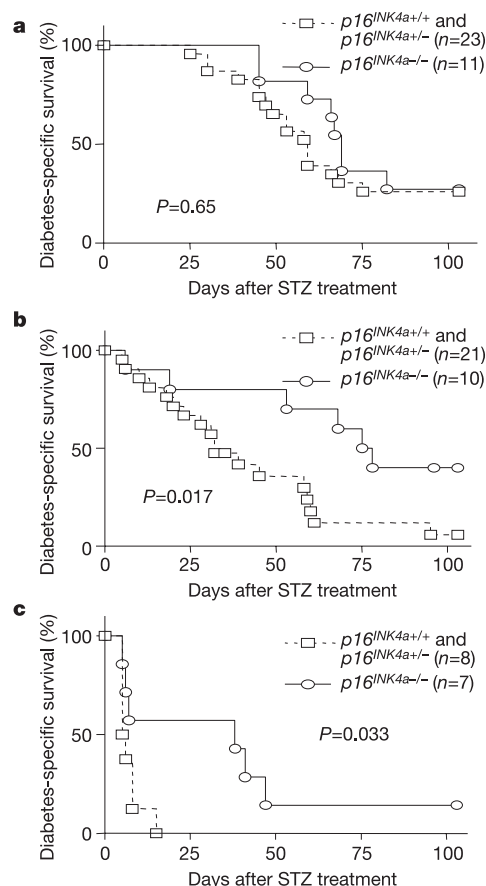


Figure 4 | Influence of $p16^{\text{INK4a}}$ expression on islet regeneration after STZ treatment. **a–c**, Diabetes-specific survival after STZ treatment of mice treated at 5–9 weeks (**a**), 9–15 weeks (**b**) or 20–28 weeks (**c**) of age. Indicated P -values were determined by log-rank test.

$p16^{INK4a}$ deficiency afforded resistance to STZ in older animals (Fig. 4b, c). After STZ injection, older $p16^{INK4a-/-}$ animals exhibited a more rapid recovery to pre-treatment weight (Fig. 3a), a higher probability of return to normo-glycaemia (Fig. 3c), and enhanced survival (Fig. 4b, c) compared with $p16^{INK4a+/+}$ littermates. Although the greatest survival differences were seen in the oldest mice, as with islet proliferation, $p16^{INK4a}$ deficiency did not entirely rescue this age-induced phenotype. Therefore, an age-associated increase in $p16^{INK4a}$ expression seems to limit the recovery of islet function after STZ ablation, but $p16^{INK4a}$ -independent mechanism(s) of islet ageing also seem to exist.

These data suggest that $p16^{INK4a}$ mediates a decline in the replicative capacity of islets associated with ageing and, in this compartment, is both a biological marker and effector of ageing. Several lines of evidence support the notion that $p16^{INK4a}$ expression also contributes to ageing in additional tissues. The expression of $p16^{INK4a}$ in most mammalian tissues strongly correlates with ageing¹ and in some tissues is attenuated by caloric restriction², which retards mammalian ageing. Furthermore, increased expression of $p16^{INK4a}$ with ageing diminishes the replicative capacities of neural and haematopoietic stem cells in a cell-autonomous fashion^{26,27}. These results from diverse systems suggest that $p16^{INK4a}$ contributes to mammalian ageing by limiting the self-renewal of regenerative cells in at least the bone marrow, brain and endocrine pancreas.

Although expression of $p16^{INK4a}$ has an age-dependent impact on islet proliferation and regeneration, the compartment in which $p16^{INK4a}$ exerts an age-promoting function is not clear. The simplest model would be that $p16^{INK4a}$ expression in β -cells directly limits their replication, which has been suggested to be the principal mechanism of β -cell production in adult mice²⁸. Alternatively, $p16^{INK4a}$ could regulate the proliferation of putative stem cells suggested to contribute to β -cell production in adult mice²⁹. It is also not clear how inhibition of cyclin D/Cdk4 activity by $p16^{INK4a}$ exerts an age-promoting function. It is possible that $p16^{INK4a}$ induces an irreversible growth arrest (senescence) of β -cells or their progenitors, as has been suggested previously³⁰. Alternatively, it is possible that $p16^{INK4a}$ expression decreases the frequency of cell cycle entry without inducing a permanent growth arrest.

Although insulin resistance is a characteristic feature of type 2 diabetes mellitus, most adults with insulin resistance do not develop type 2 diabetes mellitus. Similarly, the sharp increase in the incidence of type 2 diabetes mellitus with age cannot be solely attributed to increased insulin resistance. Recent evidence has demonstrated a decrease in β -cell mass of type 2 diabetes mellitus patients relative to matched non-diabetic controls^{31,32}. Our data indicate that increased $p16^{INK4a}$ expression with ageing contributes to a relative failure of islet proliferation. This observation suggests the possibility that type 2 diabetes mellitus, in part, results from a $p16^{INK4a}$ -induced replicative failure of islets with ageing. Further studies, however, are required to link formally senescence and type 2 diabetes mellitus in humans.

METHODS

Islets and exocrine pancreas were isolated from C57BL/6 mice (Jackson Labs) of indicated ages as described¹⁴. TaqMan analysis was performed as described² with minor modifications described in Supplementary Methods. Single-copy BAC transgenic mice overexpressing $p16^{INK4a}$ but not *Arf* or $p15^{INK4b}$ were generated using a 64-kilobase genomic clone derived from the RPCI-22 (129/SvEv) BAC library. Eleven independent founder lines were generated, at least two of which were single-copy integrants (Supplementary Fig. 2). All experiments were performed using littermate progeny from a single-copy transgenic line ($p16^{INK4a(Tg)}$) demonstrating mean ~5-fold overexpression of $p16^{INK4a}$ mRNA and protein across a panel of tissues (Supplementary Figs 2 and 3). Islet proliferation and STZ survival experiments were performed in littermate progeny from $p16^{INK4a+/+}$ intercrosses in the C57BL/6 and FVB/n backgrounds (see Supplementary Methods). For survival after STZ treatment, cohorts of littermate mice of indicated genotypes were treated with STZ (150 mg kg⁻¹ body weight). Comparisons of proliferation were performed by calculation of the Ki67 proliferative index using established methods within the Brigham and

Women's Hospital Pathology Immunohistochemical Laboratory. Insulin staining and Ki67/insulin double staining were performed as described in Supplementary Information. Detailed methodological information is available in Supplementary Methods.

Received 11 April; accepted 24 July 2006.

Published online 6 September 2006.

1. Zindy, F., Quelle, D. E., Roussel, M. F. & Sherr, C. J. Expression of the $p16^{INK4a}$ tumor suppressor versus other INK4 family members during mouse development and aging. *Oncogene* 15, 203–211 (1997).
2. Krishnamurthy, J. et al. *Ink4a/Arf* expression is a biomarker of aging. *J. Clin. Invest.* 114, 1299–1307 (2004).
3. Nielsen, G. P. et al. Immunohistochemical survey of $p16^{INK4a}$ expression in normal human adult and infant tissues. *Lab. Invest.* 79, 1137–1143 (1999).
4. Park, I. K., Morrison, S. J. & Clarke, M. F. Bmi1, stem cells, and senescence regulation. *J. Clin. Invest.* 113, 175–179 (2004).
5. Campisi, J. Cancer and ageing: rival demons? *Nature Rev. Cancer* 3, 339–349 (2003).
6. Lowe, S. W. & Sherr, C. J. Tumor suppression by *Ink4a-Arf*: progress and puzzles. *Curr. Opin. Genet. Dev.* 13, 77–83 (2003).
7. Rane, S. G. et al. Loss of Cdk4 expression causes insulin-deficient diabetes and Cdk4 activation results in β -islet cell hyperplasia. *Nature Genet.* 22, 44–52 (1999).
8. Tsutsui, T. et al. Targeted disruption of CDK4 delays cell cycle entry with enhanced $p27^{Kip1}$ activity. *Mol. Cell. Biol.* 19, 7011–7019 (1999).
9. Halvorsen, T. L., Beattie, G. M., Lopez, A. D., Hayek, A. & Levine, F. Accelerated telomere shortening and senescence in human pancreatic islet cells stimulated to divide *in vitro*. *J. Endocrinol.* 166, 103–109 (2000).
10. Kushner, J. A. et al. Cyclins D2 and D1 are essential for postnatal pancreatic β -cell growth. *Mol. Cell. Biol.* 25, 3752–3762 (2005).
11. Hino, S. et al. *In vivo* proliferation of differentiated pancreatic islet β cells in transgenic mice expressing mutated cyclin-dependent kinase 4. *Diabetologia* 47, 1819–1830 (2004).
12. Marzo, N. et al. Pancreatic islets from cyclin-dependent kinase 4/R24C (Cdk4) knockin mice have significantly increased β cell mass and are physiologically functional, indicating that Cdk4 is a potential target for pancreatic β cell mass regeneration in Type 1 diabetes. *Diabetologia* 47, 686–694 (2004).
13. Pei, X. H., Bai, F., Tsutsui, T., Kiyokawa, H. & Xiong, Y. Genetic evidence for functional dependency of $p18^{INK4c}$ on Cdk4. *Mol. Cell. Biol.* 24, 6653–6664 (2004).
14. Montana, E., Bonner-Weir, S. & Weir, G. C. Beta cell mass and growth after syngeneic islet cell transplantation in normal and streptozotocin diabetic C57BL/6 mice. *J. Clin. Invest.* 91, 780–787 (1993).
15. Fernandes, A. et al. Differentiation of new insulin-producing cells is induced by injury in adult pancreatic islets. *Endocrinology* 138, 1750–1762 (1997).
16. Uchida, T. et al. Deletion of *Cdkn1b* ameliorates hyperglycemia by maintaining compensatory hyperinsulinemia in diabetic mice. *Nature Med.* 11, 175–182 (2005).
17. Sharpless, N. E. et al. Loss of $p16^{INK4a}$ with retention of $p19^{Arf}$ predisposes mice to tumorigenesis. *Nature* 413, 86–91 (2001).
18. Sharpless, N. E., Ramsey, M. R., Balasubramanian, P., Castrillon, D. H. & DePinho, R. A. The differential impact of $p16^{INK4a}$ or $p19^{ARF}$ deficiency on cell growth and tumorigenesis. *Oncogene* 23, 379–385 (2004).
19. Bonner-Weir, S., Trent, D. F., Honey, R. N. & Weir, G. C. Responses of neonatal rat islets to streptozotocin: limited β -cell regeneration and hyperglycemia. *Diabetes* 30, 64–69 (1981).
20. Riley, W. J., McConnell, T. J., Maclaren, N. K., McLaughlin, J. V. & Taylor, G. The diabetogenic effects of streptozotocin in mice are prolonged and inversely related to age. *Diabetes* 30, 718–723 (1981).
21. Gu, D., Arnush, M. & Sarvetnick, N. Endocrine/exocrine intermediate cells in streptozotocin-treated Ins-IFN- γ transgenic mice. *Pancreas* 15, 246–250 (1997).
22. Meng, A., Wang, Y., Van Zant, G. & Zhou, D. Ionizing radiation and busulfan induce premature senescence in murine bone marrow hematopoietic cells. *Cancer Res.* 63, 5414–5419 (2003).
23. Wang, Y., Schulte, B. A., Larue, A. C., Ogawa, M. & Zhou, D. Total body irradiation selectively induces murine hematopoietic stem cell senescence. *Blood* 107, 358–366 (2006).
24. Jacobs, J. J. & de Lange, T. Significant role for $p16^{INK4a}$ in p53-independent telomere-directed senescence. *Curr. Biol.* 14, 2302–2308 (2004).
25. Robles, S. J. & Adami, G. R. Agents that cause DNA double strand breaks lead to $p16^{INK4a}$ enrichment and the premature senescence of normal fibroblasts. *Oncogene* 16, 1113–1123 (1998).
26. Molofsky, A. V. et al. Increasing $p16^{INK4a}$ expression decreases forebrain progenitors and neurogenesis during ageing. *Nature advance online publication*, doi:10.1038/nature05091 (6 September 2006).
27. Janzen, V. et al. Stem-cell ageing modified by the cyclin-dependent kinase inhibitor $p16^{INK4a}$. *Nature advance online publication*, doi:10.1038/nature05159 (6 September 2006).
28. Dor, Y., Brown, J., Martinez, O. I. & Melton, D. A. Adult pancreatic β -cells are formed by self-duplication rather than stem-cell differentiation. *Nature* 429, 41–46 (2004).

29. Bonner-Weir, S. & Weir, G. C. New sources of pancreatic β -cells. *Nature Biotechnol.* **23**, 857–861 (2005).
30. Sone, H. & Kagawa, Y. Pancreatic β cell senescence contributes to the pathogenesis of type 2 diabetes in high-fat diet-induced diabetic mice. *Diabetologia* **48**, 58–67 (2005).
31. Butler, A. E. *et al.* β -cell deficit and increased β -cell apoptosis in humans with type 2 diabetes. *Diabetes* **52**, 102–110 (2003).
32. Yoon, K. H. *et al.* Selective β -cell loss and α -cell expansion in patients with type 2 diabetes mellitus in Korea. *J. Clin. Endocrinol. Metab.* **88**, 2300–2308 (2003).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank J. Lock, S. Alson, M. Zhang, Y. Xiong and G. Enders for advice, reagents and technical support, and R. DePinho and K. Wong for comments on the manuscript. This work was supported by grants from the Sidney Kimmel Cancer Foundation for Cancer Research, the Paul Beeson Physician Scholars program, the Ellison Medical Foundation, and the National Institutes of Health.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to N.E.S. (NES@med.unc.edu).

LETTERS

BRX mediates feedback between brassinosteroid levels and auxin signalling in root growth

Céline F. Mouchel¹, Karen S. Osmont¹ & Christian S. Hardtke¹

Brassinosteroid and auxin decisively influence plant development, and overlapping transcriptional responses to these phytohormones suggest an interaction between the two pathways^{1–3}. However, whether this reflects direct feedback or merely parallel inputs on common targets is unclear. Here we show that in *Arabidopsis* roots, this interaction is mediated by *BREVIS RADIX* (*BRX*), which is required for optimal root growth⁴. We demonstrate that the *brx* phenotype results from a root-specific deficiency of brassinosteroid and is due to reduced, *BRX*-dependent expression of a rate-limiting enzyme in brassinosteroid biosynthesis. Unexpectedly, this deficiency affects the root expression level of ~15% of all *Arabidopsis* genes, but the transcriptome profile can be restored to wild type by brassinosteroid treatment. Thus, proper brassinosteroid levels are required for the correct expression of many more genes than previously suspected. Moreover, embryonic or post-embryonic brassinosteroid application fully or partially, respectively, rescues the *brx* phenotype. Further, auxin-responsive gene expression is globally impaired in *brx*, demonstrating that brassinosteroid levels are rate-limiting for auxin-responsive transcription. *BRX* expression is strongly induced by auxin and mildly repressed by brassinolide, which means that *BRX* acts at the nexus of a feedback loop that maintains threshold brassinosteroid levels to permit optimal auxin action.

BRX has been identified through a natural loss-of-function allele in the *Arabidopsis* accession Umkirch-1 (Uk-1), and results in reduced root meristem size, reduced mature cell size, and thus reduced root growth⁴. *BRX* belongs to a conserved, plant-specific gene family that encodes proteins that are predicted to regulate transcription^{4,5}. Because *BRX* is expressed at very low levels^{4,5}, we could determine its expression pattern only with a *BRX::GUS* reporter gene (Supplementary Fig. 1a–f), which reveals *BRX* expression in the columella and the phloem vasculature throughout root and shoot. In the absence of apparent morphological shoot phenotypes in *brx* plants^{4,5}, the significance of the shoot expression is unclear. Despite the mutant phenotype, *BRX* is not expressed in the cell proliferation zone of the root meristem. However, continuous expression connecting the incipient columella and vasculature is detected in the embryo.

To determine where *BRX* expression is required for root growth, we expressed *BRX*-GFP fusion proteins under the control of heterologous promoters in *brx*^S seedlings (derived from introgression of the only available loss-of-function *brx* allele into a control background, Sav-0 (ref. 4)). Promoters that confer expression in only the root meristem or columella do not complement the *brx*^S root growth defect. In contrast, promoters that confer expression throughout the root vasculature rescue the *brx*^S phenotype as efficiently as the native *BRX* promoter (Supplementary Fig. 1g). Thus, *BRX* activity is primarily required in the vasculature.

The expression pattern suggests that the *brx* mutation indirectly influences meristem size, possibly by disturbing polar auxin transport because this results in phenotypes similar to *brx*^S (refs 6–8). Root

growth of auxin transport mutants is often resistant to auxin transport inhibitors and, indeed, *brx*^S roots are slightly resistant to the same inhibitors (Supplementary Fig. 1h). However, uptake kinetics of the endocytic tracer dye FM4-64 are normal in *brx*^S root cells (Supplementary Fig. 1i), arguing against a general defect in the auxin transport machinery⁹. Furthermore, applying small amounts of auxin does not restore growth rate or meristem size of *brx*^S roots, but restores reduced meristem size in certain *pin* mutants⁶.

To verify the normal expression of genes implicated in auxin transport, we analysed 6-day-old roots of *brx*^S and its introgression parent Sav-0 using microarray analyses. In addition, we analysed a *brx*^S line complemented by a *BRX::BRX-GFP* transgene⁴ to identify expression differences resulting from residual Uk-1 loci other than *BRX*. Of the 22,746 gene signatures assayed, the expression of 4,195 significantly differs (>2-fold, $P \leq 0.01$) between *brx*^S and Sav-0 (Fig. 1a; Supplementary Table 1). Only 189 of these expression differences are due to residual Uk-1 DNA (Fig. 1b, c). Most of the remaining 4,006 genes are strongly (>3-fold) under- or over-expressed in *brx*^S (Fig. 1d). Thus, loss of *BRX* function (*brx*) alters the root expression levels of ~15% of *Arabidopsis* genes, although these differences might partly reflect secondary changes that are due to the reduced growth rate.

Of the ~4,000 differentially expressed genes, only a few have been implicated in auxin transport. These include the *PIN-FORMED* auxin efflux facilitators *PIN3* and *PIN4* (refs 6, 7), and the *Arabidopsis thaliana* P-glycoprotein auxin influx facilitator *AtPGP4* (ref. 8). All three genes are involved in organizing auxin flow at the root tip and their loss of function reduces primary root growth^{6–8}. Therefore, the reduced *brx*^S root growth possibly results from disturbed auxin transport, although this could be a secondary defect. Another misexpressed gene that could explain the *brx*^S phenotype is *CONSTITUTIVE PHOTOMORPHOGENESIS AND DWARF* (*CPD*). *CPD* is highly underexpressed in *brx*^S roots (Fig. 1e) and encodes a rate-limiting enzyme for biosynthesis of brassinolide, the major active brassinosteroid in *Arabidopsis*^{10,11}. Loss of *CPD* function (*cpd*) results in severe dwarfism, including reduced root growth^{10,12}. Thus, *brx*^S roots are possibly brassinolide deficient and we tested whether this could cause the observed transcriptome alterations by performing another set of microarray analyses. In these experiments, the minimal shoot system of the 4-day-old seedlings used (seedlings of this age were used in these experiments for technical reasons) masked only 934 out of the 4,006 expression differences between 6-day-old roots of *brx*^S and Sav-0—mostly those of lower magnitude (Fig. 1f). Strikingly, a 3-hour treatment of *brx*^S seedlings with 5 nM brassinolide restores normal expression levels of 2,965 of the 3,072 differentially expressed genes, including all genes involved in auxin transport (Fig. 1g). This rescue is even more complete (3,046 out of 3,072) when compared with brassinolide-treated controls, because brassinolide affects transcription of some genes in Sav-0 (Fig. 1h).

The accurate restoration of expression levels in the absence of

¹Department of Plant Molecular Biology, Biophore Building, University of Lausanne, CH-1015 Lausanne, Switzerland.

apparent morphological changes during the 3-hour brassinolide treatment suggests that the disruption of gene expression in *brx^S* is primarily because of suboptimal brassinosteroid levels. Importantly, *CPD* is among the very few genes that show no expression response to the brassinolide treatment (Supplementary Fig. 2). In contrast, expression of other brassinosteroid biosynthesis or signalling genes is restored and reacts to brassinolide application as described^{13,14}. Therefore, our data suggest that *CPD* expression requires *BRX* expression.

If the brassinolide effect on the *brx^S* transcriptome is physiologically relevant, then brassinolide treatment should also rescue the *brx^S* phenotype. Indeed, *brx^S* root growth is significantly enhanced in the presence of 1–5 nM brassinolide (Fig. 2a). This effect is also observed in an independent introgression of the *brx* allele into the Col-0 background⁴ (*brx^C*), but not in complemented *brx^S* lines (Fig. 2b), suggesting that it is linked to the *brx* loss-of-function mutation. This partial rescue results from restored mature cell length (Fig. 2c), rather than restored meristem size. Because *BRX* is expressed in the meristem of embryonic roots, we also investigated the effect of brassinolide on *brx^S* embryogenesis. To this end, developing siliques were dipped once into brassinolide solution at 1 day after fertilization and the progeny were assayed for their root phenotype. Whereas brassinolide treatment did not affect meristem morphology or root growth of Sav-0 seedlings, meristem size was fully rescued in *brx^S* seedlings (Fig. 2d). Moreover, embryonic brassinolide treatment also restored growth rate and cell elongation (Fig. 2e, f), consistent with the positive correlation between meristem size and mature cell length in wild-type plants^{15,16}.

This rescue is embryogenesis-specific, because imbibing untreated

brx^S seeds in brassinolide solution before germination does not restore root growth. Further, roots of *brx^S* seedlings from brassinolide-treated siliques grown in the presence of brassinolide behave like Sav-0 roots: growth and cell elongation are slightly inhibited (Fig. 2e). However, embryonic brassinolide treatment is not sufficient to rescue growth rate at later stages of development (Fig. 2g). In particular, the roots lack the characteristic growth acceleration¹⁵ observed in the controls after 10–12 days. Therefore, our assays suggest that *BRX*, and by inference brassinolide, is required during embryogenesis to establish proper root meristem size, and post-embryonically for optimal root growth. The idea that brassinosteroids are required for root growth is also supported by the reduced growth rate of *bri1* roots (Supplementary Fig. 3), which are defective in the principal brassinosteroid receptor. Our results also suggest that root meristem size is initially determined during embryogenesis. Finally, consistent with the idea that *brx^S* brassinosteroid deficiency results from reduced *CPD* expression, *BRX*-independent expression of *CPD* in *brx^S* under the constitutive CaMV 35S promoter enhances *brx^S* root growth (Fig. 2h). This rescue is more efficient than post-embryonic brassinolide application (to ~60% versus ~50% of control root length), but still partial, probably because the 35S promoter does not express well during embryogenesis.

The strong effect of brassinolide application on the *brx^S* transcriptome contrasts with its limited, rather weak, effects on wild-type transcriptomes^{1,2,17,18}. However, synergistic effects of brassinolide and auxin application on transcription have been described^{1,2,19}, and brassinolide is thought to act at least partly through regulating auxin signalling^{19–21}. Furthermore, brassinosteroid biosynthesis

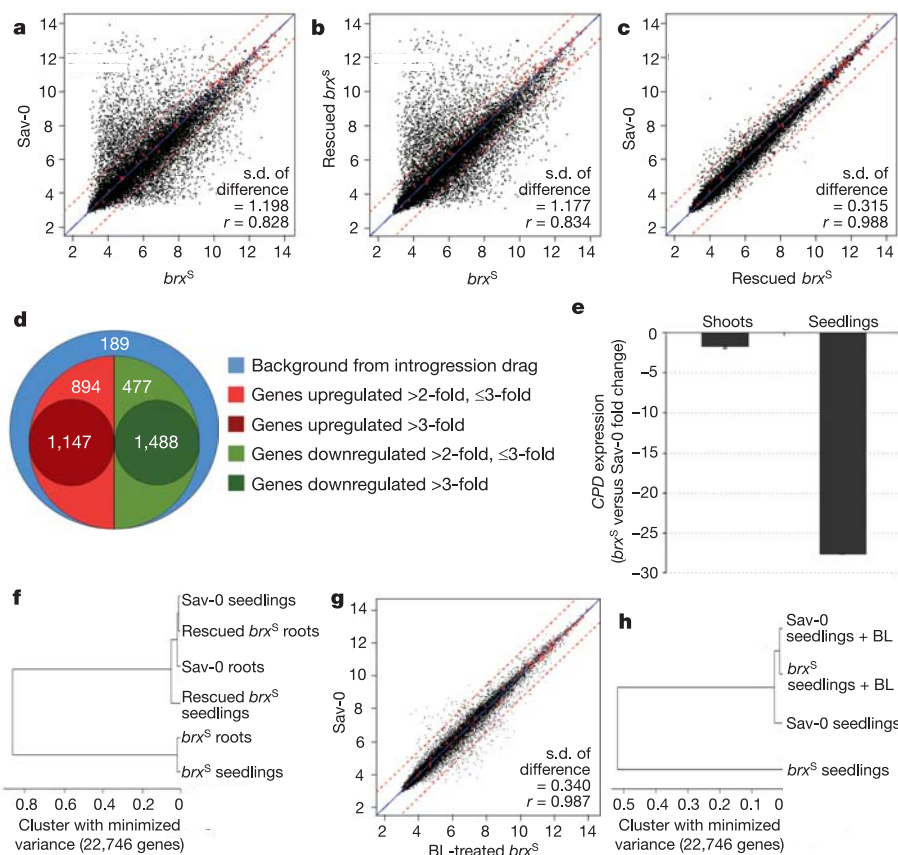


Figure 1 | Microarray analyses. **a–c**, Scatter plots of expression level differences between roots of: **a**, *brx^S* and Sav-0; **b**, *brx^S* and *brx^S* complemented by *BRX::BRX-GFP* (rescued *brx^S*; ref. 4); and **c**, Sav-0 and rescued *brx^S*. The standard deviation (s.d.) of differences and correlation coefficients (r) are given. **d**, The number of constitutively misexpressed genes in *brx^S* roots. **e**, qPCR analysis of relative *CPD* expression in *brx^S*, as

compared with Sav-0. **f**, Cluster analysis (arbitrary units) of microarray data for roots and seedlings. **g**, Scatter plot of expression level differences between Sav-0 seedlings, and *brx^S* seedlings treated with brassinolide (BL). **h**, Cluster analysis of microarray data for Sav-0 and *brx^S* seedlings, with or without BL treatment. Error bars are s.e.m.

inhibitor treatment prevents auxin induction of the prototypical auxin-responsive reporter gene *DR5::GUS* (ref. 21). Therefore, we tested auxin response in *brx^S* via microarray analyses. Notably, with two exceptions, none of the 290 genes that responded to auxin treatment in *Sav-0* responded significantly in *brx^S* (Supplementary Table 1). In addition, the basic expression level of *DR5::GUS* is reduced in *brx^S* roots, especially in the vasculature, as is auxin-responsiveness (Fig. 3a). However, vascular expression and auxin response can be largely restored by brassinolide treatment. Similar results are obtained if auxin is replaced by its analogue 1-naphthalene acetic acid, which can enter cells by diffusion, implying that the reduced auxin response is not a problem of auxin uptake. Rather, our results suggest that optimal brassinosteroid levels are rate-limiting for auxin-induced transcriptional responses.

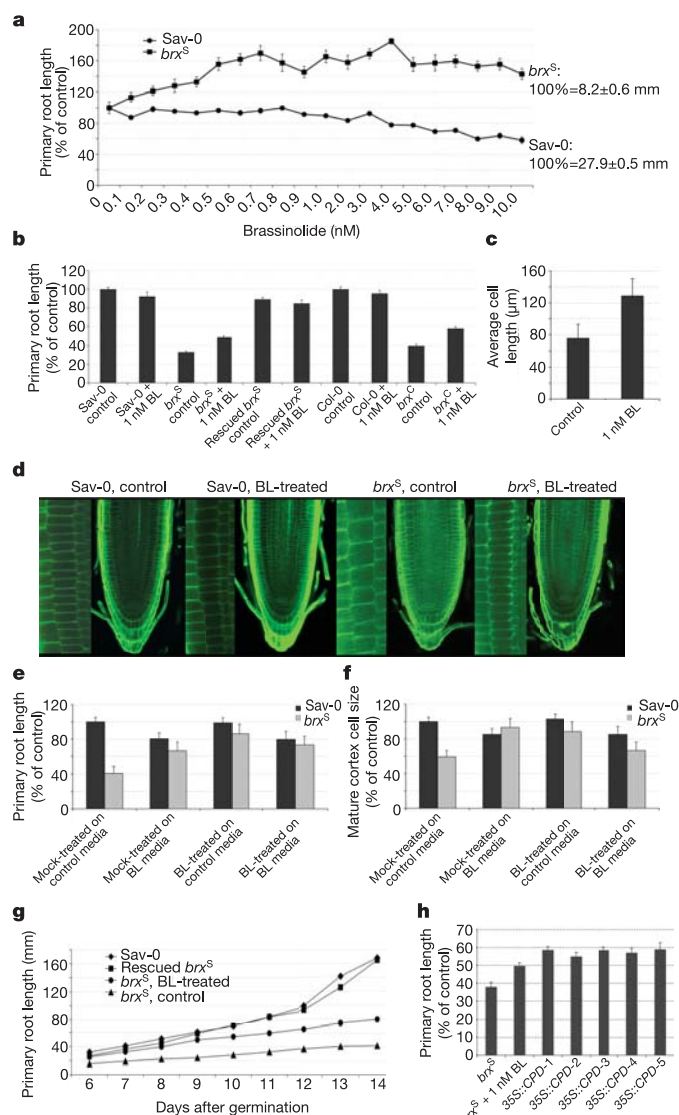


Figure 2 | Complementation of *brx^S* root phenotypes by brassinolide.

a, Root growth on increasing amounts of BL. **b**, Response (shown by relative root length) of different introgression lines and rescued *brx^S* to BL. **c**, Cortical cell length in *brx^S* roots. **d**, Size and cell proliferation rescue of *brx^S* meristems (4 days after germination) by embryonic BL application (confocal images, $\times 25$). Corresponding magnified ($\times 40$) cortical cell files are shown to the left. **e**, Relative root length of seedlings derived from mock-treated or BL-treated siliques. **f**, Cortical cell size of roots described in (**e**). **g**, Growth curve of *brx^S* roots from BL-treated siliques. **h**, Relative root length of transgenic *brx^S* seedlings constitutively expressing CPD. Error bars are s.e.m.

Interestingly, the *BRX* promoter contains predicted binding sites for brassinosteroid- and auxin-controlled transcription factors (Supplementary Fig. 4)^{18,22}. Indeed, *BRX* transcription is strongly induced by auxin treatment of young seedlings (Fig. 3b). In contrast, brassinolide mildly represses *BRX* expression (Fig. 3b). Therefore, *BRX* should be under feedback control. Indeed, in *brx^S*, *BRX* expression is reduced several-fold and auxin induction of *BRX* expression is strongly diminished (Fig. 3c, d).

Interaction between the auxin and brassinosteroid signalling pathways has been suggested on the basis of transcriptional responses. However, whether this interaction involves regulation at the level of hormone biosynthesis, or whether it converges at target genes or earlier, remains controversial^{12,17,19–21}. Our results suggest that brassinosteroid biosynthesis and auxin signalling are connected

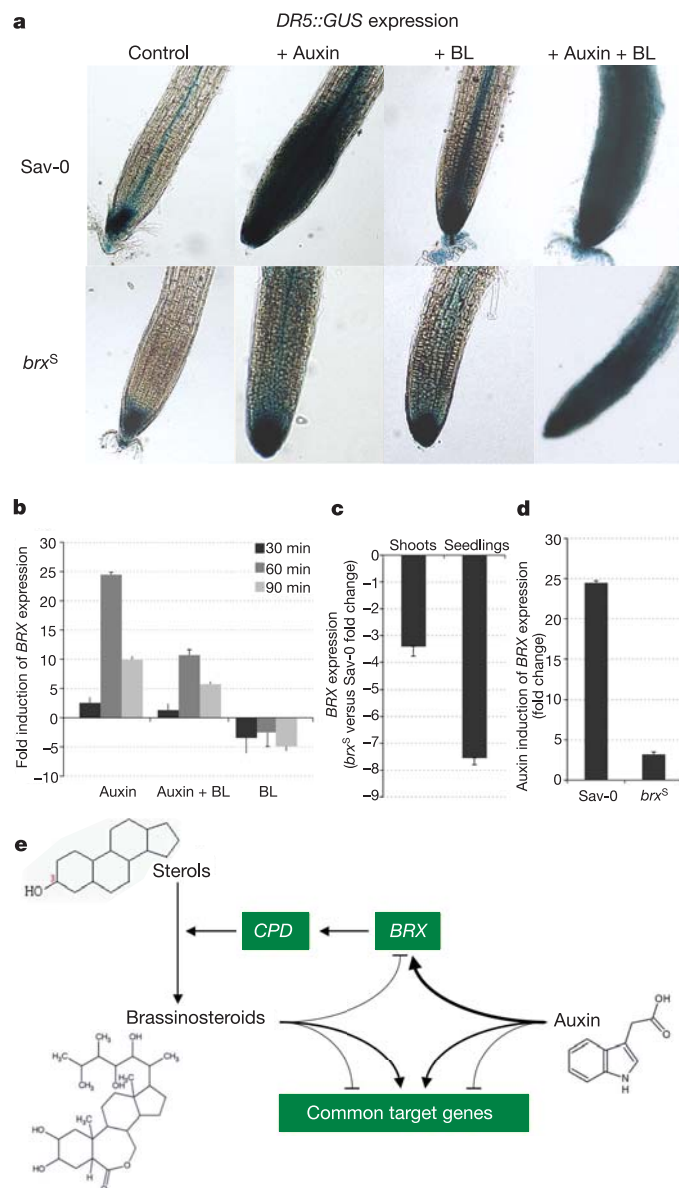


Figure 3 | Auxin-induced transcription in *brx^S*. **a**, *DR5::GUS* expression in representative *Sav-0* and *brx^S* roots. Blue staining indicates reporter activity after mock treatment (control), auxin treatment, BL treatment or combined treatment. **b**, qPCR of *BRX* expression in young seedlings in response to auxin and/or BL treatment. **c**, qPCR analysis of relative *BRX* expression as compared with *Sav-0*. **d**, qPCR analysis of *BRX* auxin-induction in *Sav-0* and *brx^S* seedlings. **e**, Proposed feedback loop between BL biosynthesis and auxin signalling in root growth. Error bars are s.e.m.

through a feedback loop, which involves *BRX* (Fig. 3e) and is required for optimal root growth, both embryonically and post-embryonically. Because *BRX* activity profoundly influences meristem size and branching pattern of the primary root⁴, its modulation could serve to coordinate growth at the level of the root system. In the proposed feedback loop, *BRX* maintains *CPD* expression to keep brassinosteroid biosynthesis above a critical threshold. This threshold level permits optimal auxin action, in turn maintaining *BRX* expression at the level required for proper *CPD* activity. The instability of *BRX* protein (Supplementary Fig. 5) emphasizes the importance of this feedback for *BRX* activity. Whether a similar feedback loop operates in the shoot remains to be determined. In the meantime, *brx*^S can serve as a useful tool to decipher further details of the brassinosteroid pathway and its interaction with other hormones in root development.

METHODS

Plant material. Seedlings were grown on culture media as described⁵. For transient hormone treatments, seedlings were transferred into liquid culture. *DR5::GUS* was introduced into the *Sav-0* and *brx*^S backgrounds by crossing. Mutants of *bri1* were obtained from the *Arabidopsis* Biological Resources Center. T-DNA constructs were generated and transformed into *brx*^S plants according to standard procedures^{4,5}. Transgene activity was assayed and verified as described⁴. Promoter sequences were chosen according to published expression patterns^{23–28}. Root length was assayed at 8–9 days after germination.

GUS staining. GUS reporter activity was visualized according to standard procedures⁴. Six independent *BRX::GUS* lines were analysed in detail. For analysis of the *DR5::GUS* reporter hormone response, 7-day-old seedlings were treated with either mock solution, 1 μ M IAA or 10 μ M 1-naphthalene acetic acid for 1 h, 5 nM brassinolide for 3 h, or 5 nM brassinolide for 3 h supplemented with 1 μ M IAA after 2 h.

Microarray analyses. Labelling and hybridization of ATH1 DNA arrays (Affymetrix) was performed according to the manufacturer's instructions. For each genotype, tissue or treatment, two biological replicate experiments were performed. Intensity values were normalized with the RMA method²⁹. For analysis of hormone responses, 4-day-old seedlings were treated with either mock solution, 1 μ M IAA for 1 h, or 5 nM brassinolide for 3 h.

Embryonic brassinolide treatment. Fertilized flowers (stage 6.10; ref. 30) were dipped into mock solution or 5 nM brassinolide once.

Quantitative real-time PCR. Quantitative real-time polymerase chain reaction (qPCR) was performed on a Stratagene Mx3000P instrument, as described⁵. The relative amount of each amplicon compared with a control amplicon of the elongation factor gene *EF1 α* was used to calculate fold changes between different conditions. For analysis of *BRX* expression in response to hormone treatments, 8-day-old seedlings were treated with either mock solution, 10 μ M IAA, 1 μ M brassinolide, or 10 μ M IAA and 1 μ M brassinolide. All qPCR data represent the average of three biological replicate experiments.

Received 8 June; accepted 24 July 2006.

- Goda, H. *et al.* Comprehensive comparison of auxin-regulated and brassinosteroid-regulated genes in *Arabidopsis*. *Plant Physiol.* **134**, 1555–1573 (2004).
- Nemhauser, J. L., Mockler, T. C. & Chory, J. Interdependency of brassinosteroid and auxin signaling in *Arabidopsis*. *PLoS Biol.* **2**, E258 (2004).
- Zurek, D. M., Rayle, D. L., McMorris, T. C. & Clouse, S. D. Investigation of gene expression, growth kinetics, and wall extensibility during brassinosteroid-regulated stem elongation. *Plant Physiol.* **104**, 505–513 (1994).
- Mouchel, C. F., Briggs, G. C. & Hardtke, C. S. Natural genetic variation in *Arabidopsis* identifies *BREVIS RADIX*, a novel regulator of cell proliferation and elongation in the root. *Genes Dev.* **18**, 700–714 (2004).
- Briggs, G. C., Mouchel, C. F. & Hardtke, C. S. Characterization of the plant-specific *BREVIS RADIX* gene family reveals limited genetic redundancy despite high sequence conservation. *Plant Physiol.* **140**, 1306–1316 (2006).
- Blilou, I. *et al.* The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots. *Nature* **433**, 39–44 (2005).
- Friml, J., Wiśniewska, J., Benková, E., Mendgen, K. & Palme, K. Lateral relocation of auxin efflux regulator PIN3 mediates tropism in *Arabidopsis*. *Nature* **415**, 806–809 (2002).
- Terasaka, K. *et al.* PGP4, an ATP binding cassette P-glycoprotein, catalyzes auxin transport in *Arabidopsis thaliana* roots. *Plant Cell* **17**, 2922–2939 (2005).

- Paciorek, T. *et al.* Auxin inhibits endocytosis and promotes its own efflux from cells. *Nature* **435**, 1251–1256 (2005).
- Szeker, M. *et al.* Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in *Arabidopsis*. *Cell* **85**, 171–182 (1996).
- Tanaka, K. *et al.* Brassinosteroid homeostasis in *Arabidopsis* is ensured by feedback expressions of multiple genes involved in its metabolism. *Plant Physiol.* **138**, 1117–1125 (2005).
- Mussig, C., Fischer, S. & Altmann, T. Brassinosteroid-regulated gene expression. *Plant Physiol.* **129**, 1241–1251 (2002).
- Shimada, Y. *et al.* Organ-specific expression of brassinosteroid-biosynthetic genes and distribution of endogenous brassinosteroids in *Arabidopsis*. *Plant Physiol.* **131**, 287–297 (2003).
- Bancos, S. *et al.* Regulation of transcript levels of the *Arabidopsis* cytochrome P450 genes involved in brassinosteroid biosynthesis. *Plant Physiol.* **130**, 504–513 (2002).
- Beemster, G. T. & Baskin, T. I. Analysis of cell division and elongation underlying the developmental acceleration of root growth in *Arabidopsis thaliana*. *Plant Physiol.* **116**, 1515–1526 (1998).
- Beemster, G. T. & Baskin, T. I. *STUNTED PLANT 1* mediates effects of cytokinin, but not of auxin, on cell division and expansion in the root of *Arabidopsis*. *Plant Physiol.* **124**, 1718–1727 (2000).
- Goda, H., Shimada, Y., Asami, T., Fujioka, S. & Yoshida, S. Microarray analysis of brassinosteroid-regulated genes in *Arabidopsis*. *Plant Physiol.* **130**, 1319–1334 (2002).
- Vert, G., Nemhauser, J. L., Geldner, N., Hong, F. & Chory, J. Molecular mechanisms of steroid hormone signaling in plants. *Annu. Rev. Cell Dev. Biol.* **21**, 177–201 (2005).
- Wang, S., Tiwari, S. B., Hagen, G. & Guilfoyle, T. J. AUXIN RESPONSE FACTOR7 restores the expression of auxin-responsive genes in mutant *Arabidopsis* leaf mesophyll protoplasts. *Plant Cell* **17**, 1979–1993 (2005).
- Nakamura, A. *et al.* Brassinolide induces *IAA5*, *IAA19*, and *DR5*, a synthetic auxin response element in *Arabidopsis*, implying a cross talk point of brassinosteroid and auxin signaling. *Plant Physiol.* **133**, 1843–1853 (2003).
- Nakamura, A. *et al.* *Arabidopsis* Aux/IAA genes are involved in brassinosteroid-mediated growth responses in a manner dependent on organ type. *Plant J.* **45**, 193–205 (2006).
- Leyser, H. M. Molecular genetics of auxin signaling. *Annu. Rev. Plant Biol.* **53**, 377–398 (2002).
- Friml, J. *et al.* AtPIN4 mediates sink-driven auxin gradients and root patterning in *Arabidopsis*. *Cell* **108**, 661–673 (2002).
- Sabatini, S. *et al.* An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. *Cell* **99**, 463–472 (1999).
- Siebert, T., Hauser, M. T., Seifert, G. J. & Luschig, C. PROPORZ1, a putative *Arabidopsis* transcriptional adaptor protein, mediates auxin and cytokinin signals in the control of cell proliferation. *Curr. Biol.* **13**, 837–842 (2003).
- Tsugeki, R. & Fedoroff, N. V. Genetic ablation of root cap cells in *Arabidopsis*. *Proc. Natl Acad. Sci. USA* **96**, 12941–12946 (1999).
- Birnbaum, K. *et al.* A gene expression map of the *Arabidopsis* root. *Science* **302**, 1956–1960 (2003).
- Himanen, K. *et al.* Auxin-mediated cell cycle activation during early lateral root initiation. *Plant Cell* **14**, 2339–2351 (2002).
- Irizarry, R. A. *et al.* Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4**, 249–264 (2003).
- Boyes, D. C. *et al.* Growth stage-based phenotypic analysis of *Arabidopsis*: a model for high throughput functional genomics in plants. *Plant Cell* **13**, 1499–1510 (2001).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank T. Guilfoyle for the *DR5::GUS* reporter line; the DNA Array Facility of the University of Lausanne for assistance with microarray experiments; and J. MacDonald Chambers-Petétôt for assistance with sectioning and microscopy. C.F.M. was supported by the Canton de Vaud, Switzerland. This work was funded by a grant from the Swiss National Science Foundation awarded to C.S.H.

Author Contributions C.F.M. contributed all data except those for Fig. 2c and 2h, and Supplementary Figs 1i and 3, contributed by K.S.O. All authors were involved in the design of the research and the writing of the manuscript.

Author Information The microarray experiments have been deposited in the Array Express Database under accession numbers E-MEXP-635 and M-MEXP-637. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to C.S.H. (christian.hardtke@unil.ch).

LETTERS

Centrosome polarization delivers secretory granules to the immunological synapse

Jane C. Stinchcombe¹, Endre Majorovits², Giovanna Bossi¹, Stephen Fuller² & Gillian M. Griffiths¹

Cytotoxic T lymphocytes (CTLs) destroy virally infected and tumorigenic cells by releasing the contents of specialized secretory lysosomes—termed ‘lytic granules’—at the immunological synapse formed between the CTL and the target¹. On contact with the target cell, the microtubule organizing centre of the CTL polarizes towards the target^{2,3} and granules move along microtubules in a minus-end direction towards the polarized microtubule organizing centre. However, the final steps of secretion have remained unclear. Here we show that CTLs do not require actin or plus-end microtubule motors for secretion, but instead the centrosome moves to and contacts the plasma membrane at the central supramolecular activation cluster of the immunological synapse. Actin and IQGAP1 are cleared away from the synapse, and granules are delivered directly to the plasma membrane. These data show that CTLs use a previously unreported mechanism for delivering secretory granules to the immunological synapse, with granule secretion controlled by centrosome delivery to the plasma membrane.

Lytic granule secretion occurs in a specialized secretory domain of the immunological synapse⁴, which lies next to the central supramolecular activation complex (cSMAC)^{5,6} where T-cell receptors involved in target cell recognition localize. Both the secretory domain and the cSMAC are surrounded by the peripheral SMAC (pSMAC), which contains proteins involved in cell adhesion (Fig. 1a). The mechanisms involved in delivering granules to the secretory domain have not been characterized. Therefore, we examined the roles of the actin and tubulin cytoskeletons in granule delivery to the immunological synapse. Expression of actin fused to green fluorescent protein (actin-GFP) in CTLs reveals that labelled actin is completely cleared from the region of the immunological synapse where granules release their contents during conjugation of CTLs to target cells (Fig. 1b, c). This indicates that actin is not directly involved in the final stages of exocytosis in CTLs, raising the possibility that granules are delivered directly to the secretory domain via microtubules. Previous studies have shown two sets of microtubules within CTLs extending towards the immunological synapse: short microtubules running between the polarized microtubule organizing centre (MTOC) and the plasma membrane with plus ends towards the synapse, and long microtubules that curve past the synapse contacting the plasma membrane *en route* to their minus ends at the MTOC^{7,8}. Thus, granules might be delivered to the synapse in a plus-end direction using the short microtubules, or a minus-end direction using longer microtubules that curve past the synapse (Fig. 1d).

Rab-interacting lysosomal protein (RILP) recruits dynein through interaction with Rab7 on late endosomes/lysosomes⁹. Overexpression of RILP prevents plus-end-directed movement and causes clustering of lysosomes around the MTOC¹⁰. To distinguish the roles of plus-end- and minus-end-directed transport in the delivery of granules, we overexpressed RILP tagged with GFP (GFP-RILP) in CTLs to block plus-end-directed movement on microtubules. We observed

that RILP overexpression induces clustering of granules around the MTOC in CTLs, demonstrating that plus-end-directed granule movement by kinesins is impaired (Fig. 1e–h). To determine the effect of RILP overexpression on secretion, we isolated CTL clones in which all cells express GFP-RILP (Fig. 1i) and determined their ability to kill target cells using a cytotoxicity assay. CTLs expressing GFP-RILP killed targets with the same efficiency as mock-infected cells or cells expressing GFP alone (Fig. 1j), indicating that plus-end-directed motors are not required for granule secretion and that minus-end-directed movement along microtubules to the MTOC is sufficient for transport of granules to the synapse. Therefore, we decided to examine the precise positioning of the MTOC during lytic granule secretion.

As our studies indicated that most microtubules originate from the pericentriolar area of the centrosomes, we analysed the position

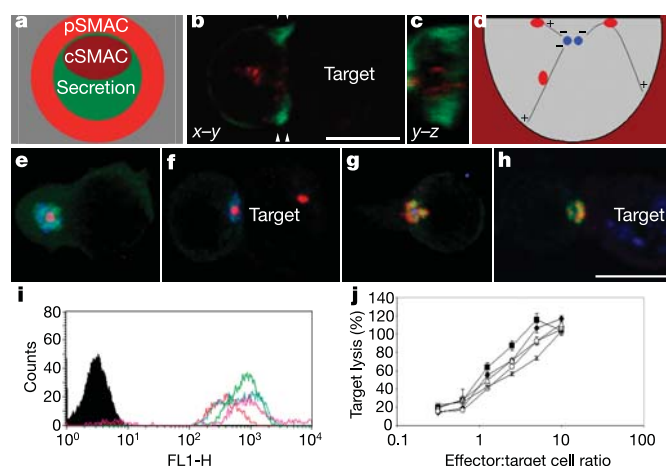


Figure 1 | Minus-end microtubule transport motors are sufficient for transport of lytic granules to the secretory site during target cell killing. **a**, *En face* view of the organization of the CTL immunological synapse. **b**, **c**, Single *x–y* confocal section (**b**) and *en face* (*y–z*) reconstruction (**c**) of a human CTL, expressing actin-GFP (green) and with CD63-labelled granules (red), conjugated to a target cell. **d**, Organization of microtubules (thin black lines) with lytic granules (red), relative to the immunological synapse (thick black line) and centrioles (blue). **e–h**, Projected serial confocal sections through human CTLs, expressing GFP-RILP (green), shown alone (**e**, **g**) or conjugated to targets (**f**, **h**), and labelled with markers of the centrosome (γ -tubulin is red in **e**, **f**, but blue in **g**, **h**) and granule markers (cathepsin D, blue in **e**, **f**; perforin, red in **g**, **h**). **i**, Fluorescence-activated cell sorting (FACS) profile showing GFP expression (FL1-H) of mock-infected (filled histogram) human CTLs and four CTL clones expressing GFP-RILP (unfilled histograms). **j**, Killing assay using mock-infected CTLs (diamonds), GFP-(squares) or GFP-RILP-(triangles, circles, crosses) expressing CTL clones. Error bars show standard deviation from the mean. Scale bars for **b**, **c** and **e–h**, 10 μ m.

¹Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE, UK. ²Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford OX3 7BN, UK.

of the centrosomes and centrioles in CTL–target conjugates by both immunofluorescence and electron microscopy (Fig. 2). Labelling with antibodies against the centriole marker, centrin, revealed that in some conjugates the centrosome contacts the plasma membrane with one centriole in front and towards the membrane (Fig. 2a; Supplementary Movie 1). Previous studies have shown that on target cell encounter, the MTOC of the CTL is dynamic and scans the immunological synapse⁷. Fixation of samples during conjugation of CTLs with targets captures individual conjugates at different stages of interaction within single samples. We examined the position of the centrosome in such samples using antibodies against γ -tubulin or acetylated tubulin, to label the centrosome, and intercellular adhesion molecule 1 (ICAM-1), to define the plasma membranes of both the CTL and target. We found that the position of the CTL centrosome varied such that 27% were unpolarized, 21% were polarized towards the contact site but distal from the plasma membrane, and 35% showed the centrosome close to, but not contacting, the plasma membrane. Notably, in the remaining 17% of conjugates the centrosome was closely associated with the CTL membrane at the contact site, demonstrating that at some point during the interaction the centrosome contacts the plasma membrane.

Electron microscopy of thin sections taken through the contact site of conjugates in which the centrosome and granules are polarized confirms that, in some cells, the centrosome is closely associated with the membrane (Fig. 2b–h). Microtubules radiate out from the polarized centrosomal area, running both away from the plasma membrane into the cell, and also parallel to and just under the cell surface immediately adjacent to the polarized centrioles (Fig. 2e–h).

Lytic granules are associated with the microtubules and are brought into contact with the plasma membrane close to the polarized centrosomes (Fig. 2e). The Golgi complex, which associates with microtubules and the MTOC¹¹, is also directed to the plasma membrane in cells with tightly polarized centrioles (Fig. 2e–h), providing a mechanism for the targeted transport of newly synthesized proteins to the membrane. Three-dimensional reconstruction of the synapse by electron tomography confirms the close association of the centrosome and granules with the plasma membrane, and reveals that microtubule ends can be detected associated with the plasma membrane only outside the area of centrosomal polarization (Supplementary Fig. 1; Supplementary Movies 2 and 3). Together, these images indicate that, on contact with a target, the centrosome not only polarizes towards the target but also transiently contacts the CTL plasma membrane within the immunological synapse. In this way, granules can be delivered directly to the plasma membrane as they reach the polarized centrosome.

To determine the exact site of centrosome polarization within the immunological synapse, we labelled CTL–target conjugates with markers against the pSMAC and cSMAC (Fig. 3a–c). The secretory domain was identified by the site of docked granules (Fig. 3d–f) and/or the location of the secretory cleft (shown by an indentation in the target cell membrane filled with secreted granzyme A; Fig. 3g, h). Three-dimensional reconstruction of confocal images taken through CTL–target conjugates was used to localize the position of centrin- or γ -tubulin-labelled centrioles within the synapse. Notably, we found that the centrosome always localizes to the same place within the immunological synapse. In 100% of conjugates analysed ($n = 67$), the centrosome contacts the plasma membrane on the edge of the

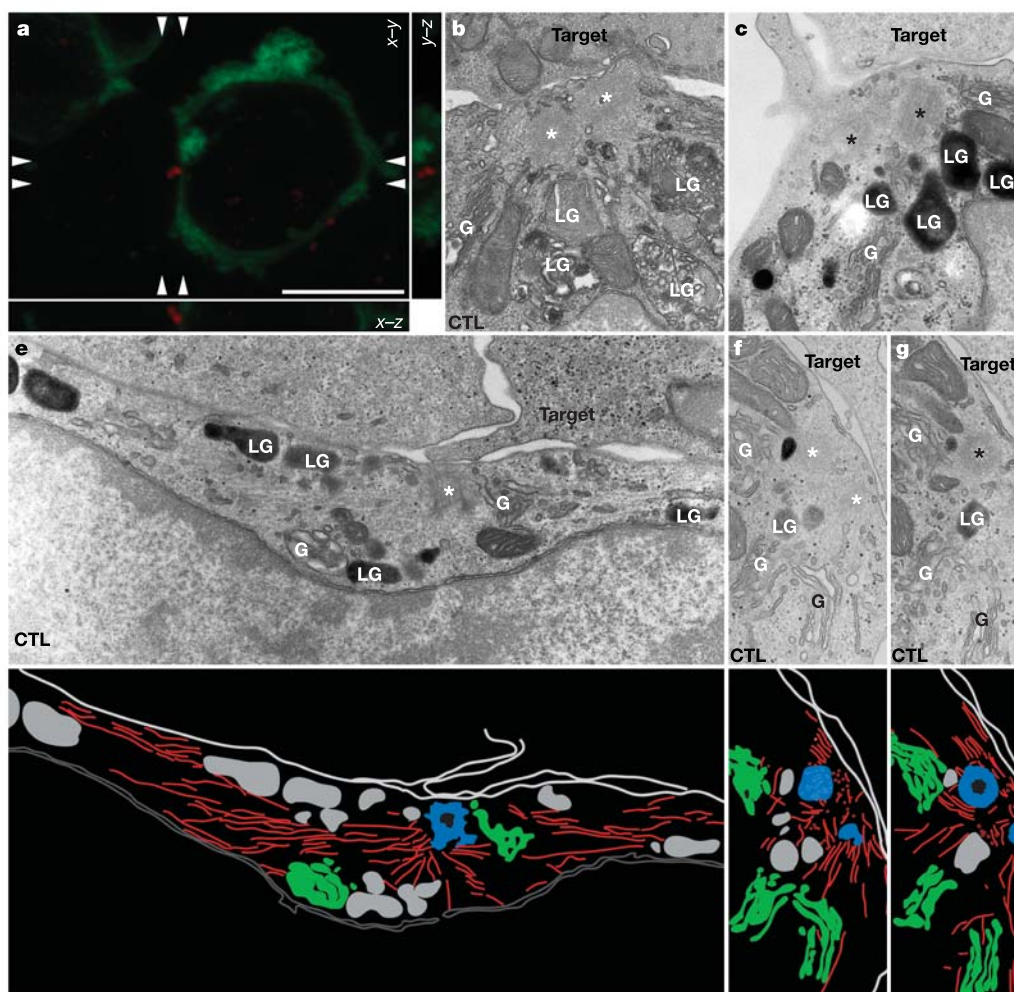


Figure 2 | The centrosome contacts the plasma membrane during target-cell killing. **a**, Mouse CTL–target conjugate identifying CTL centrioles (centrin, red) and target membrane (H2D^d, green) shown as either a projection of serial confocal sections (x – y), or reconstructions through planes indicated by arrowheads (y – z (*en face*) and x – z). Scale bar, 10 μ m. **b–h**, Electron micrograph sections through mouse (**b**) and human (**c–h**) CTLs conjugated to targets with cartoons of **e–h** given below showing centrioles (asterisk in the electron micrograph; blue in the cartoon), Golgi (G; green, cartoon) lytic granules (LG; light grey, cartoon), microtubules (red, cartoon), plasma membrane (white, cartoon) and nuclear membrane (dark grey, cartoon). Scale bar for **b–h**, 0.5 μ m.

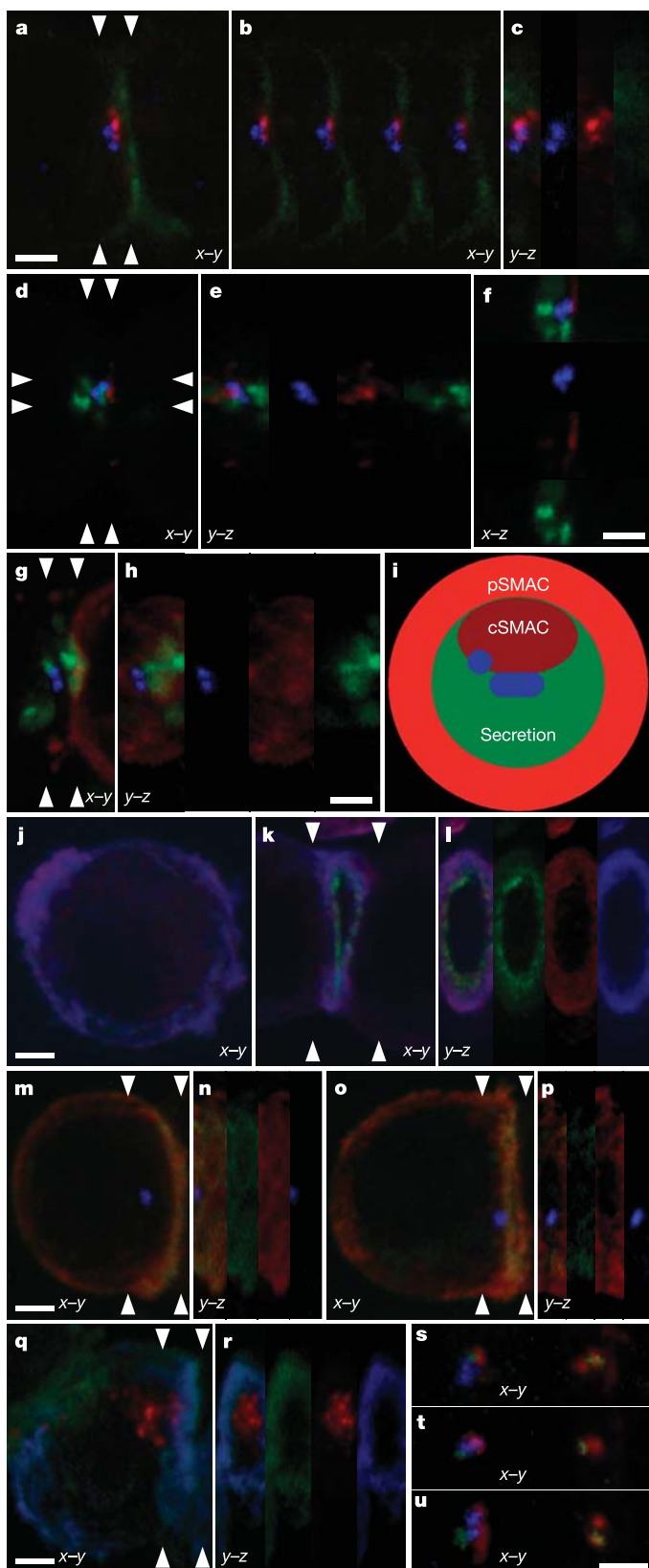
Lck-labelled cSMAC (Fig. 3a–c; Supplementary Movie 4). The polarized centrioles are surrounded by granules that dock with the plasma membrane in the area surrounding the site of centriole association (Fig. 3d–f; Supplementary Movie 5). *En face* images in the y – z plane across the contact site and reconstructed from serial z -sections (Fig. 3e–h) reveal that the centrosome polarizes to a site on

one edge of the CSMAC, surrounded by the secretory domain (defined by the docked granules and the granzyme-A-loaded secretory cleft). In summary, these images show that the centrioles contact the plasma membrane between the cSMAC and the secretory domain of the immunological synapse (Fig. 3i).

This raises the question as to what might control centriole polarization in CTLs. Previous studies have implicated the GTP-binding protein Cdc42 in the control of MTOC reorientation and reorganization of the microtubule network in T cells, because it is activated on T-cell-receptor engagement¹², and the dominant-negative form blocks both actin and MTOC polarization in CD4 T lymphocytes¹³. Cdc42 also has a role in controlling MTOC positioning by promoting microtubule plus-end tethering at the plasma membrane¹⁴. Therefore, we examined the localization of the Cdc42 effector protein IQGAP1, which forms part of a complex of proteins linking the plus ends of microtubules to the actin cortex^{14,15}. IQGAP1 labelling overlaps with that of actin in the cortex of unpolarized CTLs (Fig. 3j; Supplementary Movie 6), and is cleared from the immunological synapse along with actin, outside the adhesion ring shown by CD11a localization (Fig. 3k, l; Supplementary Movie 7). The centrosome is distal from the synapse when actin and IQGAP1 cover the synapse (Fig. 3m, n; Supplementary Movie 8), and is only found in contact with the plasma membrane when actin and IQGAP1 have cleared (Fig. 3o, p; Supplementary Movie 9). IQGAP1 is cleared when granules are delivered to the contact site (Fig. 3q, r; Supplementary Movie 10). The association of IQGAP1 with actin provides a mechanism whereby the plus ends of microtubules tethered to the actin cytoskeleton could be cleared from the synapse during actin reorganization. The resulting forces on the microtubule network might then pull the MTOC forward to contact the plasma membrane, consistent with previous observations on microtubule movement in CTLs⁷. Taken together with our finding that microtubules are cleared from the area of contact, our observations support a link between actin reorganization and centrosome polarization.

Our data reveal an unusual secretory mechanism used by CTLs during which the centrosome contacts the plasma membrane and delivers secretory granules to the immunological synapse. Centrosome polarization to the plasma membrane has also been observed during cytokinesis^{16,17}, polarity determination in *Caenorhabditis elegans*¹⁸, and cilia formation^{19,20}. In both cilia formation and cytokinesis, the mother centriole contacts the plasma membrane. Furthermore, maternal-associated proteins Odf-2²¹ and centriolin are required for primary cilia formation²² and abscission during cytokinesis¹⁷, respectively. To determine whether centrosome polarization in CTLs might be related to cilia formation and cytokinesis, we asked whether there was a preference for mother or daughter centriole to contact the plasma membrane during CTL polarization

Figure 3 | Centrosomal polarization and IQGAP1 and actin clearance at the immunological synapse. Confocal images of mouse CTL (left) and target cell (right) conjugates (a–h, k–t) or a CTL alone (j) shown as projections of serial x – y sections (a, d, g, j, k, m, o, q, s–u), sequential, single x – y sections (b) and y – z (*en face*; c, e, h, l, n, p, r) or x – z (f) reconstructions through planes indicated by arrowheads. c, e, f, h, l, n, p, r, are shown as merged and single fluorophore images. a–c, Centrioles (centrin, blue), cSMAC (Lck, red) and pSMAC (ICAM-1, green). d–f, Centrosome (γ -tubulin, blue), cSMAC (Lck, red) and lytic granules (granzyme A, green). g, h, Centrioles (centrin, blue), lytic granules (granzyme A, green) and target membrane (H2D^d, red). i, Cartoon of *en face* (y – z) view of the synapse showing centriole position (blue). j–p, pSMAC (CD11a, green), IQGAP1 (red) and either actin (blue, j–l) or centrosomes (γ -tubulin, blue, m–p). q, r, IQGAP1 (green), lytic granules (granzyme A, red) and actin (blue). s–u, Polarized centriole pairs (γ -tubulin, blue (left panels) or acetylated tubulin, red (right panels)) showing mother centriole (cenexin, green) in front (s), behind (t) or beside (u) the daughter centriole at the contact site (red, Lck (left) or PKC- θ (right)). Scale bars, 2.5 μ m.



using antibodies against cenexin to identify the mother centriole. Quantification of three-dimensional reconstructions of 47 conjugates showed a random orientation of centrioles with the mother centriole leading in 36% of conjugates (Fig. 3s), the daughter leading in 40% (Fig. 3t), and the two centrioles side by side in the remaining 24% (Fig. 3u). This indicates that the mechanism of transient centrosome polarization during secretion from CTLs is distinct from the more stable centrosome polarization seen in cytokinesis and cilia formation.

In this paper, we show that CTLs use a novel mechanism of secretion in which the centrosome transiently contacts the plasma membrane at the cSMAC and directly delivers lytic granules to the immunological synapse. We propose that centrosomal polarization might be driven by the reorganization of the actin cytoskeleton, clearing the plus ends of microtubules from the area of contact and pulling the centrosome towards the plasma membrane. This unusual mechanism of secretion can be quickly terminated as the centrosome retracts from the plasma membrane, providing CTLs with a precise mechanism for eliminating virally infected and tumorigenic targets.

METHODS

Antibodies. Lytic granules were labelled using mouse anti-human perforin (δ G9; Pharmingen), mouse anti-human CD63 (H5B6; Developmental Studies Hybridoma Bank), rabbit anti-cathepsin D (Upstate Biotechnology) and rat anti-mouse granzyme A (clone 7.1; ref. 23) antibodies. Centrosome components were identified using mouse antibodies against γ -tubulin or acetylated tubulin, rabbit anti-centrin or anti- γ -tubulin (Sigma-Aldrich) and mouse anti-cenexin²¹. Fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse CD11a (Sigma-Aldrich) or rat anti-mouse ICAM-1 (Serotec) were used to detect the pSMAC, and mouse anti-Lck (Chemicon International) or anti-PKC- θ (Upstate Biotechnology) were used to detect the cSMAC. Rabbit anti-actin (Sigma-Aldrich) and mouse monoclonals against IQGAP1 (BD-Transduction Laboratories), mouse H2D⁴ and human CD3 (UCHT1; Pharmingen) were also used. FITC-, Cy3- and Cy5-conjugated secondary antibodies, absorbed against cross-reactive species, were obtained from the Jackson ImmunoResearch Laboratories.

Human CTL, lentiviral transduction, nucleofection and cytotoxicity assays. Human CD8-positive CTLs were transfected with actin-GFP using a nucleofector (Amaxa), or transduced with RILP-GFP or GFP alone using lentiviral vectors. Cytotoxicity against P815 target cells was assayed by redirected lysis using the Cytotox 96 (Promega) assay as described in Supplementary Information.

Generation and conjugation of mouse CTLs. Mouse anti-allogeneic CTLs were generated by stimulation of spleens from C57/BL6 mice with irradiated splenocytes from BALB/c mice as described in Supplementary Information.

Immunofluorescence. Mouse or human CTL–target conjugates were prepared, fixed in -20°C methanol for 5 min and stained with antibodies, as described in Supplementary Information. Images were obtained on the BioRad 2000 confocal microscope and three-dimensional reconstructions were generated using MetaMorph 6.24R.

Electron microscopy. For electron microscopy, CTLs were incubated overnight with horse radish peroxidase (HRP) to load the endocytic pathway and processed using diaminobenzidine (DAB) cytochemistry to allow lytic granules to be identified. All processing for electron microscopy and tomography is described in Supplementary Information.

Received 18 May; accepted 11 July 2006.

1. Stinchcombe, J., Bossi, G. & Griffiths, G. M. Linking albinism and immunity: the secrets of secretory lysosomes. *Science* **305**, 55–59 (2004).
2. Geiger, B., Rosen, D. & Berke, G. Spatial relationships of microtubule-organizing centers and the contact area of cytotoxic T lymphocytes and target cells. *J. Cell Biol.* **95**, 137–143 (1982).

3. Kupfer, A. & Dennert, G. Reorientation of the microtubule-organizing center and the Golgi apparatus in cloned cytotoxic lymphocytes triggered by binding to lysable target cells. *J. Immunol.* **133**, 2762–2766 (1984).
4. Stinchcombe, J. C., Bossi, G., Booth, S. & Griffiths, G. M. The immunological synapse of CTL contains a secretory domain and membrane bridges. *Immunity* **15**, 751–761 (2001).
5. Monks, C. R., Freiberg, B. A., Kupfer, H., Sciaky, N. & Kupfer, A. Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature* **395**, 82–86 (1998).
6. Lin, J., Miller, M. J. & Shaw, A. S. The c-SMAC: sorting it all out (or in). *J. Cell Biol.* **170**, 177–182 (2005).
7. Kuhn, J. R. & Poenie, M. Dynamic polarization of the microtubule cytoskeleton during CTL-mediated killing. *Immunity* **16**, 111–121 (2002).
8. Poenie, M., Kuhn, J. & Combs, J. Real-time visualization of the cytoskeleton and effector functions in T cells. *Curr. Opin. Immunol.* **16**, 428–438 (2004).
9. Jordens, I., Marsman, M., Kuijl, C. & Neefjes, J. Rab proteins, connecting transport and vesicle fusion. *Traffic* **6**, 1070–1077 (2005).
10. Jordens, I. *et al.* The Rab7 effector protein RILP controls lysosomal transport by inducing the recruitment of dynein–dynactin motors. *Curr. Biol.* **11**, 1680–1685 (2001).
11. Allan, V. J., Thompson, H. M. & McNiven, M. A. Motoring around the Golgi. *Nature Cell Biol.* **4**, E236–E242 (2002).
12. Cannon, J. L. & Burkhardt, J. K. The regulation of actin remodeling during T-cell–APC conjugate formation. *Immunol. Rev.* **186**, 90–99 (2002).
13. Stowers, L., Yelon, D., Berg, L. J. & Chant, J. Regulation of the polarization of T cells toward antigen-presenting cells by Ras-related GTPase CDC42. *Proc. Natl Acad. Sci. USA* **92**, 5027–5031 (1995).
14. Fukata, M. *et al.* Rac1 and Cdc42 capture microtubules through IQGAP1 and CLIP-170. *Cell* **109**, 873–885 (2002).
15. Lansbergen, G. & Akhmanova, A. Microtubule plus end: a hub of cellular activities. *Traffic* **7**, 499–507 (2006).
16. Piel, M., Nordberg, J., Euteneuer, U. & Bornens, M. Centrosome-dependent exit of cytokinesis in animal cells. *Science* **291**, 1550–1553 (2001).
17. Gromley, A. *et al.* Centriolin anchoring of exocyst and SNARE complexes at the midbody is required for secretory-vesicle-mediated abscission. *Cell* **123**, 75–87 (2005).
18. Cowan, C. R. & Hyman, A. A. Centrosomes direct cell polarity independently of microtubule assembly in *C. elegans* embryos. *Nature* **431**, 92–96 (2004).
19. Sorokin, S. P. Reconstructions of centriole formation and ciliogenesis in mammalian lungs. *J. Cell Sci.* **3**, 207–230 (1968).
20. Bornens, M. Centrosome composition and microtubule anchoring mechanisms. *Curr. Opin. Cell Biol.* **14**, 25–34 (2002).
21. Lange, B. M. & Gull, K. A molecular marker for centriole maturation in the mammalian cell cycle. *J. Cell Biol.* **130**, 919–927 (1995).
22. Ishikawa, H., Kubo, A., Tsukita, S. & Tsukita, S. Odf2-deficient mother centrioles lack distal/subdistal appendages and the ability to generate primary cilia. *Nature Cell Biol.* **7**, 517–524 (2005).
23. Fruth, U. *et al.* A novel serine proteinase (HuTSP) isolated from a cloned human CD8⁺ cytolytic T-cell line is expressed and secreted by activated CD4⁺ and CD8⁺ lymphocytes. *Eur. J. Immunol.* **17**, 1625–1633 (1987).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank J. Neefjes for the RILP complementary DNA, B. Imhof for actin-GFP, A. Thrasher for lentiviral vectors, M. Simon for anti-granzyme A antibodies, and K. Gull for anti-cenexin antibodies. J. Kaufman, P. A. van der Merwe and K. Gull provided many helpful discussions. This research was funded by the Wellcome Trust. Funding for the Dunn School Bio-imaging facility was provided by the Edward Abraham and Wellcome Trusts. G.M.G. is the recipient of a Royal Society Wolfson Research Merit Award.

Author Contributions J.C.S. carried out all experiments except for RILP analysis (G.B.), tomographic reconstruction (E.M. and S.F.) and CTL culture (G.M.G.). J.C.S. and G.M.G. analysed the data and wrote the manuscript with contributions from all authors.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to G.M.G. (gillian.griffiths@path.ox.ac.uk).

LETTERS

Structural insights into yeast septin organization from polarized fluorescence microscopy

Alina M. Vrabioiu¹ & Timothy J. Mitchison¹

Septins are polymerizing GTPases¹ that function in cortical organization and cell division^{2–4}. In *Saccharomyces cerevisiae* they localize at the isthmus between the mother and the daughter cells, where they undergo a transition from a non-dynamic hourglass-shaped assembly⁵ to two separate rings, at the onset of cytokinesis^{6,7}. Septins form filaments as pure protein⁸ and *in vivo*⁹, but the filament organization within the hourglass and ring structures is controversial^{9,10}. Here, we use polarized fluorescence microscopy¹¹ of orientationally constrained green fluorescent protein to determine septin filament organization and dynamics in living yeast. We found that the hourglass is made of filaments aligned along the yeast bud neck. During the transition from hourglass to rings the filaments rotate through 90° in the membrane plane and become circumferential. These data resolve a long-standing controversy in the field and provide strong evidence that septins have a mechanical function in cell division.

S. cerevisiae septins can be followed *in vivo* by using green fluorescent protein (GFP) fusions^{6,7,12}. Polarized fluorescence microscopy^{13–17} can be used to determine the average direction of the resulting dipole ensemble¹⁸, and thus the organization of GFP-tagged septins, provided that GFP has a known, restricted orientation relative to the septin protein^{19,20}. Although the structure of the septin proteins is unknown, both Cdc3 and Cdc12 septins are predicted to contain a carboxy-terminal α -helix that assembles into a coiled coil²¹. In addition, GFP contains an amino-terminal α -helix^{22–24}. Joining these α -helices after suitable truncation should result in a longer α -helix and should therefore constrain the orientation of the fluorophore (Fig. 1a). We created septin–GFP fusions on the basis of this strategy, and replaced one of the endogenous septin alleles in diploid cells with the engineered fusion. The resulting GFP ensembles had a strongly polarized fluorescence, showing that the fluorophore is orientationally constrained and that the septin structures are ordered *in vivo*. This method of creating orientationally constrained C-terminal GFP fusion proteins should be generally applicable.

We define a microscope fixed coordinate system XYZ , with the Z -axis along the optical path and the X -axis and the Y -axis in the microscope stage plane (Fig. 1b). Our set-up includes polarizers with transmission axes parallel to X or Y in both the excitation and the emission pathways (see Methods). We also define a septin-based coordinate system xyz ; x and y are tangent to the bud-neck surface, whereas z is perpendicular to it (Fig. 1b). The septin-occupied bud-neck surface can be reasonably approximated by a hyperboloid of one sheet. Measurements are made in the different focal planes A , B and C , as indicated in Fig. 1b.

The fluorescence intensities measured with polarizers in the light path, I_{XX} and I_{YY} , reflect the projections of the GFP dipoles on the polarizers' transmission axes, the X and Y directions. (I_{XX} and I_{YY} are the intensities measured with the use of the XX and YY polarizer

configurations, respectively.) When the yeast cell is oriented with the mother–daughter axis in the stage plane (XY ; Fig. 1b), the intensity of area A reflects the x and y dipole projections, whereas the intensity of area B reflects the y and z projections. Similarly, when the yeast cell is oriented with the mother–daughter axis perpendicular to the stage, the intensity of area C reflects the z and x projections of the dipole ensemble (Fig. 1b). In general, by moving the yeast cell to different orientations relative to the fixed coordinate system XYZ , the contributions of the x , y and z components of the dipoles to the measured

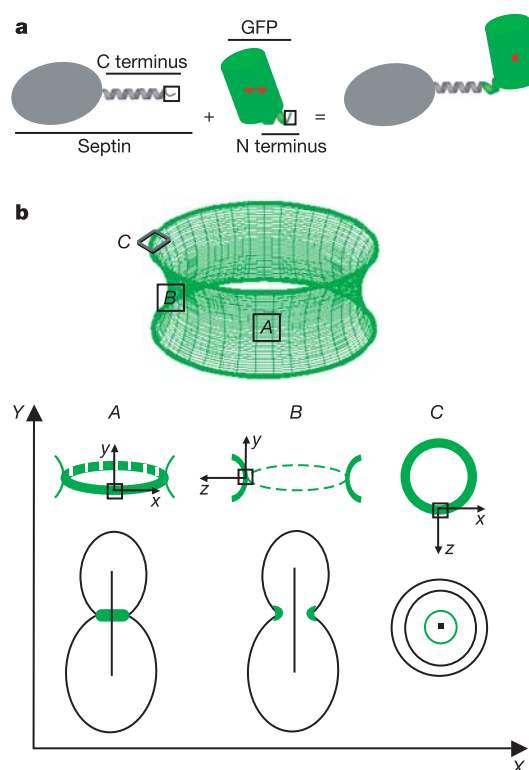


Figure 1 | The experimental system. **a**, The predicted C-terminal septin α -helix was directly linked to the N-terminal GFP α -helix by deleting the random-coil amino-acids flanking the two α -helices. The red double arrow and red dot indicate the GFP dipole in different orientations. **b**, A Mathematica-generated plot of the septin-covered surface with measurement areas A , B and C . A yeast cell is positioned within the microscope's fixed XYZ coordinate system with the mother–daughter axis either parallel (vertical black line) or perpendicular (black dot) to the stage plane (XY). The septin-based xyz coordinate system is also shown. Green, septin localization.

¹Department of Systems Biology, Harvard Medical School, 200 Longwood Avenue, Boston, Massachusetts 02115, USA.

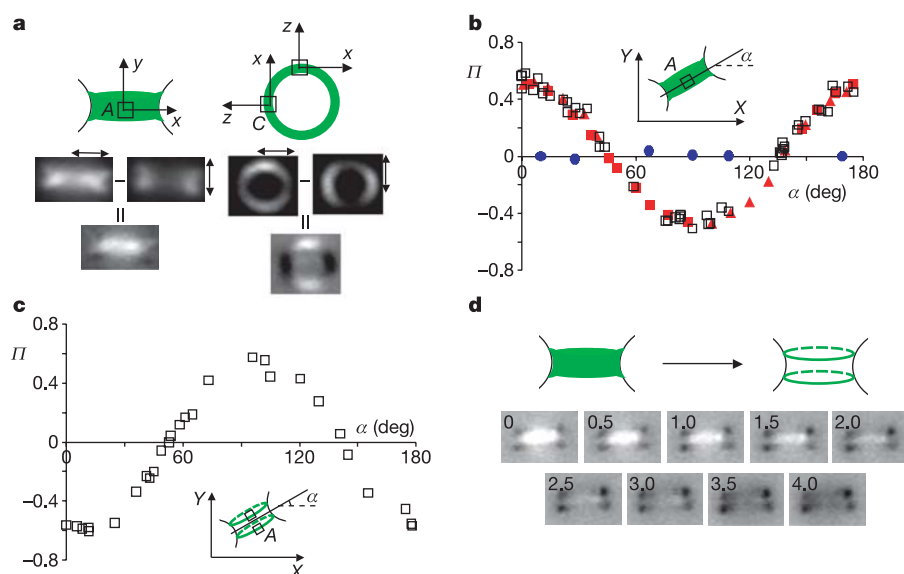


Figure 2 | The average dipole direction. **a**, The diagram shows the imaged hourglass positions and the sampled dipole projections. Below, identically scaled images acquired with parallel polarizers as indicated by the black double arrows (top) and their qualitative subtractive image (bottom) are shown. The double-headed arrows represent 1 μm . **b**, Area *A* hourglass quantifications (*II*) as a function of α . Filled red symbols (squares and triangles), data from rotating two individual yeast cells; open black squares,

population data; filled blue circles, population data from yeast expressing full-length Cdc3 fused to full-length GFP, showing isotropic fluorescence. **c**, Area *A* quantification (*II*) as a function of α for an asynchronous population that displays ring structures. **d**, Identically scaled subtractive images (*XX*–*YY*) acquired during the transition from hourglass to rings; the elapsed time (minutes) is shown on each image.

intensities can be systematically varied. From such information the average dipole direction within the *xyz* coordinate system can be determined.

Here we focus on the Cdc3 protein; a summary of the data for Cdc12 that confirm our conclusions is shown in Supplementary Fig. 1.

We first rotated individual yeast cells positioned with the mother–daughter axis in the stage plane and measured area *A* intensities (I_{XX} and I_{YY}) as a function of α , the angle that the bud-neck diameter makes with the *X*-axis. We computed $II = (I_{XX} - I_{YY}) / (I_{XX} + I_{YY})$ for each angular position (Fig. 2b, filled red symbols) and found that its maximum value (about 0.55) was obtained when $\alpha = 0$. This indicates that the average dipole projection in the *xy* plane is along the *x*-axis. We also made this measurement on the cells of a yeast population situated at random angular positions to the *X*-axis. We found that the population data (Fig. 2b, open black squares) was similar to the single-cell data (Fig. 2b, filled red symbols). Thus, septins have the same ordered, characteristic arrangement in every cell.

Yeast cells oriented with the mother–daughter axis perpendicular to the stage (Fig. 1b, C) also display anisotropic fluorescence (Fig. 2a). The regions tangent to the *X*-axis are more intense with the *XX* polarizer arrangement, whereas the regions tangent to the *Y*-axis are more intense with the *YY* polarizer arrangement. This indicates that the average dipole projection in the *zx* plane is tangent to the imaged circle, which is the *x*-axis (Fig. 2a; quantification is shown in Supplementary Fig. 2).

Because *x* is the average dipole projection in both the *xy* and *zx* planes, we conclude that *x* is the average dipole direction within the hourglass.

Septins undergo a marked change in their localization pattern at the onset of cytokinesis^{6,7}. We measured intensities of area *A* (I_{XX} and I_{YY}) as a function of α for a population of ring structures. As shown in Fig. 2c, *II* is maximum when $\alpha = 90^\circ$, indicating that the average dipole projection of the rings in the *xy* plane is along the *y*-axis. We also revealed this transition in individual yeast cells by time-lapse

imaging (Fig. 2d, and Supplementary Movie; quantifications are shown in Supplementary Fig. 3). The GFP dipoles change their orientation by 90° during the transition from hourglass to rings.

To determine the average filament direction from the average dipole direction requires a knowledge of the dipole orientation relative to the septin filament. Septin filaments can be prepared *in vitro* from purified septins, and they tend to bundle such that the filaments are aligned parallel to the bundle axis⁸. We determined *II* values for the septin bundles and plotted them as a function of α , the angle the bundle makes with the *X*-axis. *II* is maximum for $\alpha = 90^\circ$ (Fig. 3a). This measurement restricts the average dipole direction to a plane perpendicular to the filament axis. Within the hourglass structure, the average dipole direction is along the *x*-axis. For this direction to be perpendicular to the filament longitudinal axis, the filaments need to be positioned in the *yz* plane. As shown previously by electron microscopy⁹, the filaments are positioned in the membrane plane, the *xy* plane. We therefore conclude that the hourglass filaments are oriented on average along the *y*-axis, the intersection of the *xy* and *yz* planes (Fig. 3b, and Supplementary Discussion). Similarly, the ring filaments are situated in the membrane plane (*xy* plane) and are also restricted by our experiments (Figs 2c and 3a) to the *xz* plane. The ring filaments are therefore oriented on average parallel to the *x*-axis (circumferential; Fig. 3b).

Apart from the filament orientation within the bud neck, these results indicate that, *in vivo*, the filaments are rotationally constrained around their longitudinal axes. Specifically, the hourglass filaments need to be equivalently positioned in respect to the membrane. If the filaments were not rotationally constrained, they would display an isotropic pattern when observed end-on (plane C), which is not the case (Fig. 2a). In addition, the ring structures are anisotropic when the ring filaments are observed end-on (the cross-section of the rings as in the last images of Fig. 2d), indicating that the ring filaments are also rotationally constrained. These constraints might come from the preferential interaction of one side of the filament with the membrane, or from interactions between the filaments.

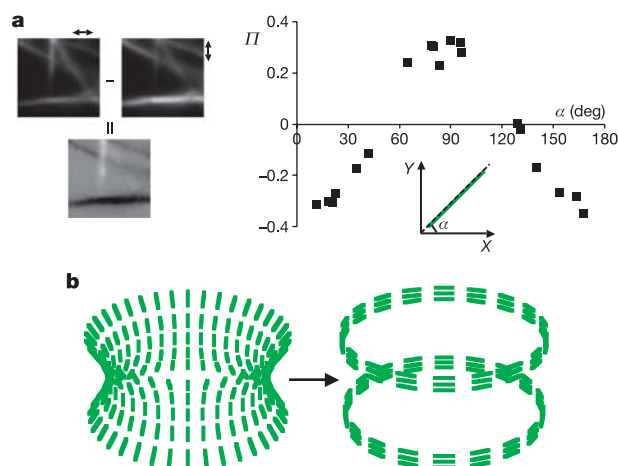


Figure 3 | The average filament direction. **a**, Left: identically scaled images of bundles assembled *in vitro* from immunopurified septin filaments, together with their qualitative subtractive image. Right: quantification of Π versus α (the angle between the bundle axis and the X-axis) for a population of bundles. The double-headed arrows represent 1 μm . **b**, The hourglass septin filaments oriented along the bud neck become circumferential in the rings.

The 90° rotation could, in principle, reflect a rotation of the Cdc3 α -helix or an orientational change of the entire septin filament. We strongly favour the latter because we observed the same dipole rotation in several different GFP fusions to both Cdc3 and Cdc12 (Supplementary Fig. 1, and data not shown), and also because electron microscopy of cortices of unroofed spheroplasts⁹ generated images that can be interpreted as two orthogonal septin organizations. We consider it unlikely that the hourglass filaments depolymerize, dissociate from the neck and repolymerize as rings for several reasons: the turnover rate measured by photobleaching is too slow^{25,26}, we observed anisotropy throughout the transition (Fig. 2d, and Supplementary Fig. 3 and Supplementary Movie) and the polymer mass is conserved (as determined by Z-series volume integrations of GFP intensities for yeast cells that undergo the transition; data not shown). We conclude that the hourglass filaments rotate through 90° in the membrane plane during the transition from hourglass to rings.

The septin rotation is a remarkable feat of molecular choreography that highlights a possible mechanical function for septins in the temporal regulation of cytokinesis. Triggered by the Tem1 small GTPase⁶, this rearrangement marks the beginning of cytokinesis. The bud-neck membrane previously occupied by septins is therefore free to ingress^{6,27} and complete the cell separation. Further evidence that will form the subject of another paper indicates that the hourglass filament array possesses sub-resolution symmetry that breaks apart during the rearrangement. This indicates that the septin filaments might be intrinsically asymmetric and capable of assembling higher-order structures (hourglass and rings) with distinct filament-filament interactions.

How do the septin filaments separate, rotate in the membrane plane and assemble into ring structures? Probably a protein modification such as dephosphorylation (ref. 25, and A.M.V. and T.J.M., unpublished *in vitro* observations) triggers their dissociation. A gradient in the stiffness of the bud neck as potentially triggered by delayed dephosphorylation of the middle of the septin scaffold compared with its sides could trigger the rotation of the filaments. However, as the middle of the hourglass is cleared of septins, the resulting rings become anisotropic in cross-section (four black spots in Fig. 2d; see also Supplementary Movie). Mechanistically, this indicates that the filaments might undergo most of the rotation at the location of the rings rather than throughout the hourglass.

Further exploration of this rearrangement will enrich our understanding of cellular mechanisms for force generation and cell division.

METHODS

Microscope set-up. A Nikon TE300 microscope set-up²⁸ with two filter wheels (Lambda 10-2; Sutter Instruments) was used. Each filter wheel (excitation and emission) contained two orthogonal polarizers that could be moved sequentially in front of the light path as controlled by Metamorph 6.3 (Molecular Devices). The polarizers were mounted in the excitation filter wheel such that the transmission axes were either horizontal (parallel to the X-axis) or vertical (parallel to the Y-axis). The polarizers in the emission filter wheel (situated just before the Hamamatsu ORCA ER camera) were parallel to the excitation polarizers, relative to the light path. Images of the same field were acquired with the horizontal horizontal (XX) and vertical vertical (YY) configurations. A Nikon 100 $\times$ Pol objective (numerical aperture 1.25) was used.

Data collection. Yeast was grown in rich medium (YPD) at room temperature (21 °C) and asynchronous populations or populations arrested with nocodazole (50 μM nocodazole in 1% dimethylsulphoxide for 2 h at room temperature) were analysed. The cells were pelleted, resuspended in water or minimal medium, and imaged on a 25% gelatin (G-9382; Sigma) pad²⁹. For imaging yeast cells with the mother-daughter axis parallel to the Z-axis, cells were mixed in a solution of 3% agarose (SeaKem Gold; Cambrex) on a microscope slide at about 40 °C, and covered with a no. 1.5 coverslip. Yeast cells were identified under transmitted light and were further imaged with the polarizers set-up at 20 °C. The average intensities and angles of particular areas were determined with Metamorph functions. A box (3 pixels $\times$ 3 pixels) was drawn on the desired area (A, B or C; Fig. 1b). The calibrated pixel dimensions for our set-up were 66 nm $\times$ 66 nm.

Received 30 March; accepted 24 July 2006.

- Field, C. M. *et al.* A purified *Drosophila* septin complex forms filaments and exhibits GTPase activity. *J. Cell Biol.* **133**, 605–616 (1996).
- Kinoshita, M. *et al.* Nedd5, a mammalian septin, is a novel cytoskeletal component interacting with actin-based structures. *Genes Dev.* **11**, 1535–1547 (1997).
- Neufeld, T. P. & Rubin, G. M. The *Drosophila* peanut gene is required for cytokinesis and encodes a protein similar to yeast putative bud neck filament proteins. *Cell* **77**, 371–379 (1994).
- Hartwell, L. H. Genetic control of the cell division cycle in yeast. IV. Genes controlling bud emergence and cytokinesis. *Exp. Cell Res.* **69**, 265–276 (1971).
- Dobbelaere, J. & Barral, Y. Spatial coordination of cytokinetic events by compartmentalization of the cell cortex. *Science* **305**, 393–396 (2004).
- Lippincott, J., Shannon, K. B., Shou, W., Deshaies, R. J. & Li, R. The Tem1 small GTPase controls actomyosin and septin dynamics during cytokinesis. *J. Cell Sci.* **114**, 1379–1386 (2001).
- Cid, V. J., Adamikova, L., Sanchez, M., Molina, M. & Nombela, C. Cell cycle control of septin ring dynamics in the budding yeast. *Microbiology* **147**, 1437–1450 (2001).
- Frazier, J. A. *et al.* Polymerization of purified yeast septins: evidence that organized filament arrays may not be required for septin function. *J. Cell Biol.* **143**, 737–749 (1998).
- Rodal, A. A., Kozubowski, L., Goode, B. L., Drubin, D. G. & Hartwig, J. H. Actin and septin ultrastructures at the budding yeast cell cortex. *Mol. Biol. Cell* **16**, 372–384 (2005).
- Byers, B. & Goetsch, L. A highly ordered ring of membrane-associated filaments in budding yeast. *J. Cell Biol.* **69**, 717–721 (1976).
- Axelrod, D. Fluorescence polarization microscopy. *Methods Cell Biol.* **30**, 333–352 (1989).
- Sheff, M. A. & Thorn, K. S. Optimized cassettes for fluorescent protein tagging in *Saccharomyces cerevisiae*. *Yeast* **21**, 661–670 (2004).
- Burghardt, T. P. Model-independent fluorescence polarization for measuring order in a biological assembly. *Biopolymers* **23**, 2383–2406 (1984).
- Dale, R. E. *et al.* Model-independent analysis of the orientation of fluorescent probes with restricted mobility in muscle fibers. *Biophys. J.* **76**, 1606–1618 (1999).
- Desper, C. R. & Kimura, I. Mathematics of the polarized-fluorescence experiment. *J. Appl. Phys.* **38**, 4225–4233 (1967).
- Inoue, S., Shimomura, O., Goda, M., Shribak, M. & Tran, P. T. Fluorescence polarization of green fluorescence protein. *Proc. Natl Acad. Sci. USA* **99**, 4272–4277 (2002).
- Axelrod, D. Carbocyanine dye orientation in red cell membrane studied by microscopic fluorescence polarization. *Biophys. J.* **26**, 557–573 (1979).
- Volkmer, A., Subramaniam, V., Birch, D. J. & Jovin, T. M. One- and two-photon excited fluorescence lifetimes and anisotropy decays of green fluorescent proteins. *Biophys. J.* **78**, 1589–1598 (2000).
- Corrie, J. E. *et al.* Dynamic measurement of myosin light-chain-domain tilt and twist in muscle contraction. *Nature* **400**, 425–430 (1999).

20. Rocheleau, J. V., Edidin, M. & Piston, D. W. Intrasequence GFP in class I MHC molecules, a rigid probe for fluorescence anisotropy measurements of the membrane environment. *Biophys. J.* **84**, 4078–4086 (2003).
21. Lupas, A., Van Dyke, M. & Stock, J. Predicting coiled coils from protein sequences. *Science* **252**, 1162–1164 (1991).
22. Yang, F., Moss, L. G. & Phillips, G. N. Jr. The molecular structure of green fluorescent protein. *Nature Biotechnol.* **14**, 1246–1251 (1996).
23. Ormo, M. *et al.* Crystal structure of the *Aequorea victoria* green fluorescent protein. *Science* **273**, 1392–1395 (1996).
24. Li, X. *et al.* Deletions of the *Aequorea victoria* green fluorescent protein define the minimal domain required for fluorescence. *J. Biol. Chem.* **272**, 28545–28549 (1997).
25. Dobbelaere, J., Gentry, M. S., Hallberg, R. L. & Barral, Y. Phosphorylation-dependent regulation of septin dynamics during the cell cycle. *Dev. Cell* **4**, 345–357 (2003).
26. Caviston, J. P., Longtine, M., Pringle, J. R. & Bi, E. The role of Cdc42p GTPase-activating proteins in assembly of the septin ring in yeast. *Mol. Biol. Cell* **14**, 4051–4066 (2003).
27. Schmidt, M., Bowers, B., Varma, A., Roh, D. H. & Cabib, E. In budding yeast, contraction of the actomyosin ring and formation of the primary septum at cytokinesis depend on each other. *J. Cell Sci.* **115**, 293–302 (2002).
28. Picart, C. & Discher, D. E. Actin protofilament orientation at the erythrocyte membrane. *Biophys. J.* **77**, 865–878 (1999).
29. Tatchell, K. & Robinson, L. C. Use of green fluorescent protein in living yeast cells. *Methods Enzymol.* **351**, 661–683 (2002).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank I. Vrabioiu and colleagues at INCDMF-CEFIN, Romania, for designing and fabricating the rotating stage used for our experiments, and M. Volles for comments. This work was supported by a National Institutes of Health grant to T.J.M.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to A.M.V. (alina_vrabioiu@student.hms.harvard.edu).

LETTERS

Ion permeation through the Na^+, K^+ -ATPase

Nicolás Reyes¹ & David C. Gadsby¹

P-type ATPase pumps generate concentration gradients of cations across membranes in nearly all cells. They provide a polar transmembrane pathway, to which access is strictly controlled by coupled gates that are constrained to open alternately, thereby enabling thermodynamically uphill ion transport (for example, see ref. 1). Here we examine the ion pathway through the Na^+, K^+ -ATPase, a representative P-type pump, after uncoupling its extra- and intracellular gates with the marine toxin palytoxin². We use small hydrophilic thiol-specific reagents³ as extracellular probes and we monitor their reactions, and the consequences, with cysteine residues introduced along the anticipated cation pathway through the pump. The distinct effects of differently charged reagents indicate that a wide outer vestibule penetrates deep into the Na^+, K^+ -ATPase, where the pathway narrows and leads to a charge-selectivity filter. Acidic residues in this region, which are conserved to coordinate pumped ions, allow the approach of cations but exclude anions. Reversing the charge at just one of those positions converts the pathway from cation selective to anion selective. Close structural homology among the catalytic subunits of Ca^{2+} -, Na^+, K^+ - and H^+, K^+ -ATPases^{4–6} argues that their extracytosolic cation exchange pathways all share these physical characteristics.

In each transport cycle, up to a hundred times a second, a single Na^+, K^+ -ATPase pump exchanges three cytoplasmic Na^+ ions for two extracellular K^+ ions and hydrolyses one molecule of ATP. Twice per cycle, the gates that allow alternate-side access both close around the bound ions, occluding them deep within the protein. Occlusion of Na^+ is linked to phosphorylation of the Na^+, K^+ -ATPase by the ATP molecule hydrolysed, and K^+ occlusion is linked to dephosphorylation, thereby ensuring vectorial ion transport. Homology models of the Na^+, K^+ -ATPase α -subunit based on crystal structures of the related Ca^{2+} -ATPase^{7,8} suggest sites for the three occluded Na^+ ions^{5,9} or two occluded K^+ ions⁵. But the routes taken by ions approaching or leaving these sites remain unclear^{10–12}.

Palytoxin disrupts the coupling between the two gates of the Na^+, K^+ pump, enabling both to sometimes be open at the same time, whereupon the toxin-bound pump becomes a cation channel¹³. Ready reversibility of palytoxin², gating of palytoxin-bound 'pump-channels' by physiological pump ligands², and accessibility of cysteine residues in those channels^{14,15} at positions expected to coordinate transported ions^{5,16} all argue that cations flow through palytoxin-bound pump-channels along a normal transport route. Palytoxin seems to stabilize an E2P-like Na^+, K^+ pump conformation (one of the two principal conformations adopted by the pump once it has been phosphorylated) with an open ion pathway¹⁷.

No crystal structure of any E2P-like state with an open ion pathway is available. We therefore generated a homology model of the Na^+, K^+ -ATPase transmembrane domain (Fig. 1) based on the MgF_4^{2-} - and thapsigargin-stabilized Ca^{2+} -ATPase structure (E2· MgF_4^{2-} form)¹⁰, even though the structure¹⁰ and functional studies¹² show that its ion pathway is closed. The model has an extracellular cavity, which is bounded on one side by transmembrane

helix 4 (TM4; Fig. 1a, b, blue) and at its bottom by the outer end of TM6 (Fig. 1a, b, green, and Supplementary Fig. 1). To probe the ion pathway, we replaced four strategically located residues (yellow) in TM4 and TM6 one by one with cysteine in ouabain-resistant, *Xenopus laevis* Na^+, K^+ -ATPase α_1 subunits, which we then expressed in *Xenopus* oocytes.

We briefly exposed outside-out membrane patches to 100 nM palytoxin (Fig. 2a–d, black arrowheads) to transform mutant Na^+, K^+ pumps into channels (100 μM ouabain precluded signals from endogenous *Xenopus* Na^+, K^+ pumps), and then tested the accessibility of each engineered cysteine to hydrophilic thiol-specific reagents of similar size³, but bearing either a positive (MTSET⁺, 2-trimethylammonium-ethyl-methanethiosulphonate, blue) or a negative (MTSES[−], 2-sulphonato-ethyl-methanethiosulphonate, red) charge. The cysteine near the rim of the cavity at the top of TM4 (in mutant E321C) reacted rapidly with both MTSET⁺ and MTSES[−]; cation current through palytoxin-bound pump-channels was decreased about threefold by MTSET⁺ (Fig. 2a, e, left) but was unaltered by MTSES[−], which nonetheless reacted because it prevented current decrease by MTSET⁺ (Fig. 2a, e, right). MTSET⁺ also reacted rapidly with a cysteine near the end of TM6 (in G805C), again decreasing cation current about threefold (Fig. 2b, e, left), whereas MTSES[−] almost doubled the current (Fig. 2b, e, right). In contrast, cation current was diminished when the cysteine at the adjacent position (in T806C) was modified by MTSET⁺ (almost completely; Fig. 2c, e, left) or MTSES[−] (by about a quarter; Fig. 2c, e, right). MTSET⁺ reaction with the deeper cysteine (in G337C) also greatly decreased current (~ 10 -fold; Fig. 2d, e, left), but MTSES[−] did not react because it neither altered current nor prevented its decrease by MTSET⁺ (Fig. 2d, e, right).

Rapid reaction with MTSET⁺ suggests that all four cysteines border the ion pathway. Because reaction of MTSES[−] with cysteines at the two outermost sites (in E321C and G805C) did not decrease current, these residues must line a vestibule wide enough to accommodate the covalent MTSES[−] adduct ($\sim 5 \times 6 \text{ \AA}$; ref. 3) without sterically preventing cation flow. But the decrease in current caused by MTSES[−] or MTSET⁺ modification of cysteine at position 806 implies narrowing of the ion pathway at this point. Reaction of MTSET⁺, but not the similarly sized MTSES[−], with the deeper cysteine at 337 suggests that MTSES[−] cannot reach that site and, thus, that a cation-selectivity filter may lie between T806 and G337.

Reaction with the neutral, but similarly bulky, reagent MTSACE (2-aminocarbonyl-ethyl-methanethiosulphonate) confirmed the different effects of MTS adducts at G805C and T806C. Current was unchanged by MTSACE reaction with G805C (Fig. 3a, green arrow), which was verified by a subsequent lack of MTSET⁺ effect. But in T806C the neutral MTSACE adduct sharply decreased cation current (~ 7 -fold; Fig. 3b, green arrow), further indicating that the pathway is narrow at position 806.

To rule out changes in pump-channel open probability as explanations for the different effects of MTS reagents in mutants G805C and T806C, we applied MTSES[−] to single palytoxin-bound

¹Laboratory of Cardiac/Membrane Physiology, The Rockefeller University, New York, New York 10021, USA.

pump-channels (Fig. 3c, d). We used MTSES⁻ because the other reagents both nearly abolished current through T806C pump-channels (Figs 2c, e, and 3b). As in the recordings from hundreds of pump-channels, MTSES⁻ made the single-channel current suddenly increase when the cysteine was at position 805 (Fig. 3c, red arrow), but suddenly decrease when it was at 806 (Fig. 3d, red arrow). Measurements at several potentials confirmed these changes in channel conductance (Fig. 3c–f). We conclude that the wide channel mouth, where MTSES⁻ and MTSACE adducts fit without slowing cation flow, becomes a narrow pore at position 806, where either adduct sterically impedes cation passage.

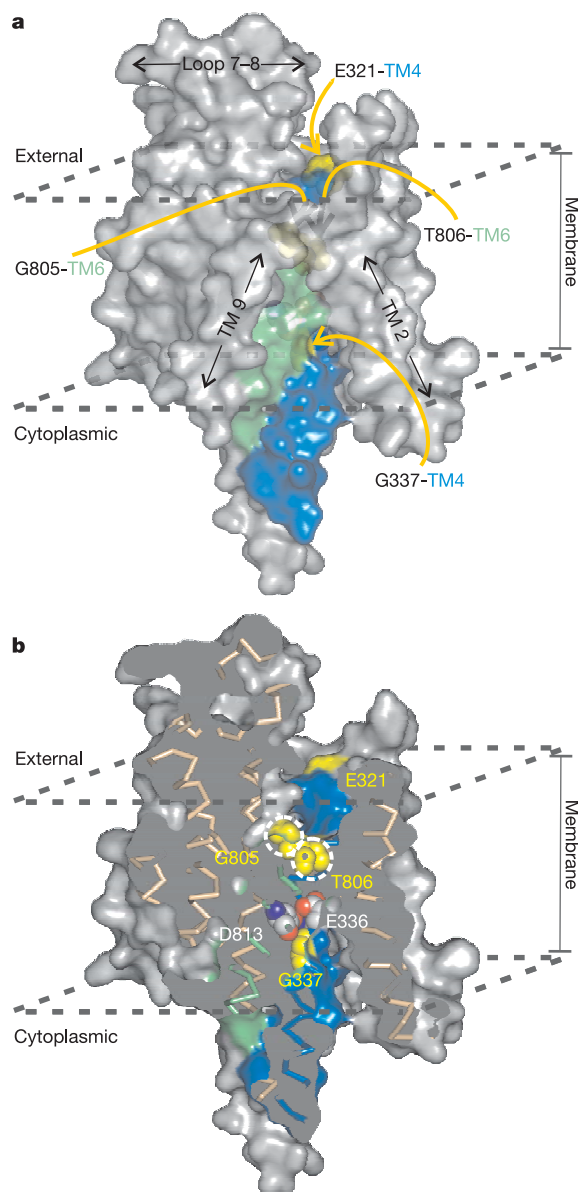


Figure 1 | Homology model of the Na⁺,K⁺-ATPase transmembrane domain. **a**, Model based on the E2·MgF₄⁻ structure of Ca²⁺-ATPase¹⁰ (PDB 1WPG). The outer solvent accessible surface is coloured grey except for TM4 (blue) and TM6 (green). Broken lines indicate approximate membrane surfaces. Four mutated residues (yellow arrows), E321 and G337 in TM4, and G805 and T806 at the top of TM6 (corresponding to Ca²⁺-ATPase residues Y294, G310, I788 and P789), are shown in yellow space-filling notation. G805 and T806 are barely visible through the pseudo-transparent surface. **b**, Vertical cut uncovers an external vestibule, with G805 and T806 near its floor, and reveals residue G337. Residues E336 and D813, equivalent to Ca²⁺-ATPase residues E309 and N796, are also indicated in Corey-Pauling-Kottun-coloured space-filling notation.

Because T806 points at TM4 in the homology model, whereas G805 points away (Supplementary Fig. 2a), orientation relative to TM4 might explain the differences in MTS effects at these positions. In support of this interpretation, the effects of the MTS reagents (MTSET⁺, MTSACE and MTSES⁻) in mutant V807C (Supplementary Fig. 2b), which is also expected to point away from TM4 (Supplementary Fig. 2a), resembled those in G805C. The failure of L802C, P803C, L804C, T808C, I809C, L810C and C811 (native cysteine) palytoxin-bound pump-channels to respond to 1 mM MTSET⁺ (data not shown) hampered further structural analysis.

If a cation-selectivity filter is located between T806 and G337 (Fig. 2), the four acidic residues in that region, E336 in TM4, E788 in TM5, and D813 and D817 in TM6 (Fig. 4a, b, and Supplementary Fig. 3), are obvious candidates to contribute to it. All four, together with the side-chain oxygen of N785, have been implicated, by mutation¹⁶ and modelling^{5,9}, in coordination of transported Na⁺ and K⁺ ions. We replaced each one with cysteine and assessed the

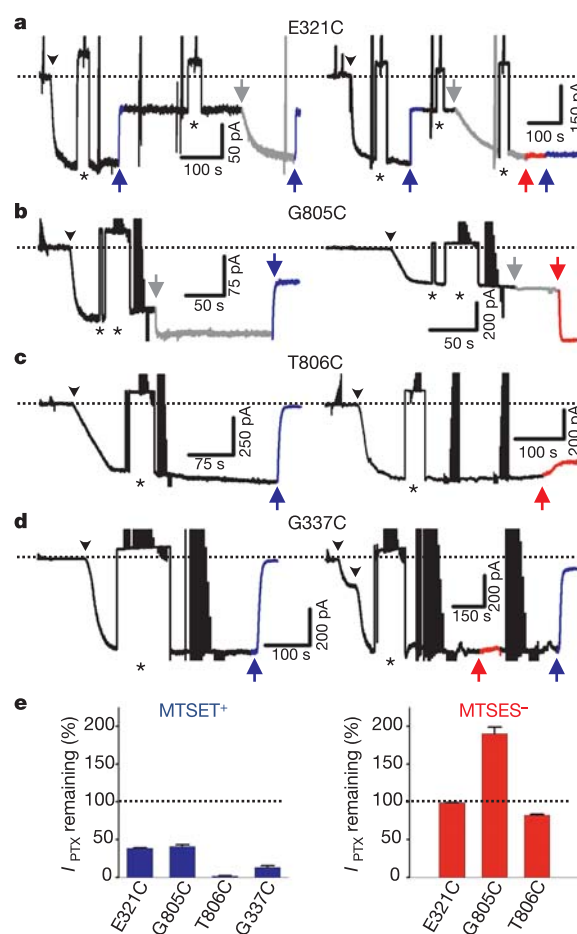


Figure 2 | Effects of MTSET⁺ and MTSES⁻ on E321C, G805C, T806C and G337C mutants. **a–d**, Current at –50 mV in outside-out patches exposed to symmetrical Na⁺ concentrations. Application of 100 nM palytoxin (black arrowheads) for 10–50 s opened hundreds of mutant Na⁺,K⁺ pump-channels, generating a macroscopic inward current (dotted line marks zero palytoxin-induced current, I_{PTX}). Temporary substitution (asterisk) of NMDG⁺ for external Na⁺ monitored patch integrity; 1 mM MTSET⁺ (blue arrows, blue traces) or MTSES⁻ (red arrows, red traces) was applied until the current was steady. Application of 10 mM dithiothreitol (grey arrows, grey traces) either reversed modification by MTSET⁺ by reducing the resulting disulphide bond (**a**), or reverted spontaneous oxidation of engineered cysteines (**b**). **e**, Percentage of I_{PTX} remaining at –50 mV after MTSET⁺ (left, E321C = 38 ± 1%, n = 3; G805C = 41 ± 2%, n = 9; T806C = 2 ± 1%, n = 8; G337C = 13 ± 3%, n = 10) or MTSES⁻ (right, E321C = 99 ± 1%, n = 2; G805C = 190 ± 9%, n = 3; T806C = 82 ± 1%, n = 3) application. All data are the mean ± s.e.m.

cation/anion selectivity of the resulting palytoxin-bound pump-channels by estimating, before and after exposure to 1 mM MTSET⁺, the permeability ratio ($P_{\text{Na}}/P_{\text{NO}_3}$) determined¹⁸ from the reversal potential shift (ΔV_{rev}) of pump-channel current on decreasing external NaNO₃ concentration. Before any cysteine was introduced, ΔV_{rev} was negative (Fig. 4c) and its magnitude matched the change in Na⁺ electrochemical equilibrium potential calculated from the Nernst equation (Fig. 4d, broken line; reproduced in Fig. 4e–g), confirming perfect cation selectivity (that is, $P_{\text{Na}}/P_{\text{NO}_3} \approx \infty$). Three cysteine substitutions, N785C ($n = 5$), E788C ($n = 4$) and D817C ($n = 2$), yielded similarly highly cation-selective pump-channels (Fig. 4e), none of which responded to 1 mM MTSET⁺. For the mutant D813C, however, cation/anion selectivity was decreased ($P_{\text{Na}}/P_{\text{NO}_3} = 8.4 \pm 0.6$, $n = 12$; Fig. 4f, black points and line), and was grossly impaired after MTSET⁺ treatment ($P_{\text{Na}}/P_{\text{NO}_3} = 1.5 \pm 0.2$, $n = 2$; Fig. 4f, blue points and line). Notably,

the TM4 mutant E336C showed greatly decreased cation/anion selectivity ($P_{\text{Na}}/P_{\text{NO}_3} = 2.8 \pm 0.03$, $n = 3$; Fig. 4g, black points and line) and was transformed from cation selective to anion selective by reaction with MTSET⁺ ($P_{\text{Na}}/P_{\text{NO}_3} = 0.2 \pm 0.01$, $n = 3$; Fig. 4g, blue points and lines). We conclude that D813 and E336 both contribute

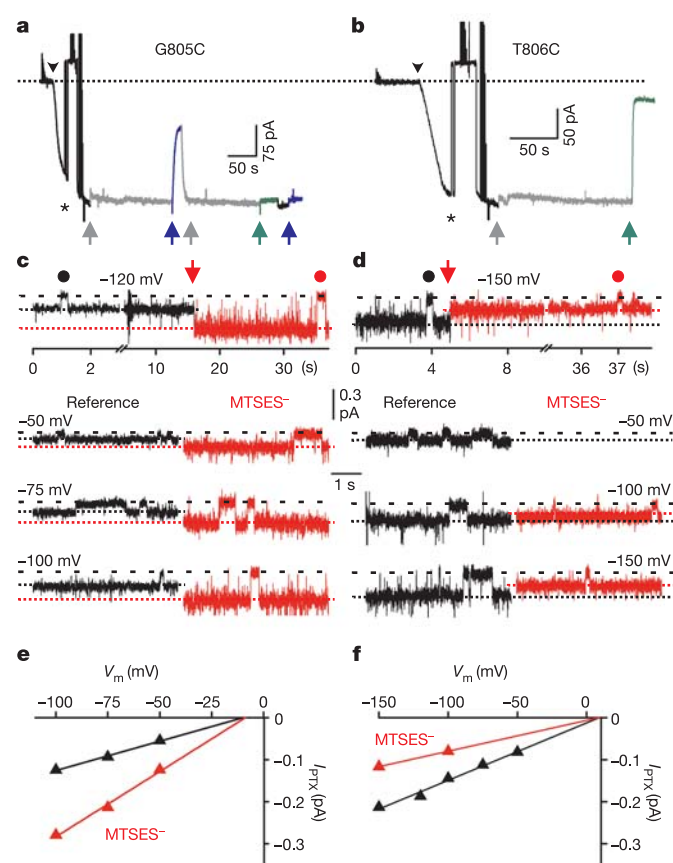


Figure 3 | Analysis of MTS-reagent action on G805C and T806C mutants. **a, b**, Currents measured under the conditions of Fig. 2. Neutral MTSACE at 1 mM (green arrows, green traces) did not alter current in G805C ($n = 3$), but greatly decreased it in T806C (% I_{PTX} remaining after MTSACE, $14 \pm 2\%$; $n = 3$) pumps. **c, d**, Outside-out patches were exposed to 100 pM palytoxin until a single G805C (**c**) or T806C (**d**) pump-channel opened. After recording channel closures (black dots), MTSET⁺ action (red arrows) on single open channels increased (G805C) or decreased (T806C) both the open-channel current (dotted lines) and the amplitudes of subsequent closure events (red dots). **e, f**, Linear fits to microscopic I_{PTX} amplitude measurements at the voltages indicated (**c, d**) yielded conductance values for G805C (**e**) of 1.4 pS before (black) and 3.1 pS after (red) MTSET⁺ application, and for T806C (**f**) of 1.4 pS before (black) and 0.7 pS after (red) MTSET⁺ application. For T806C, the % macroscopic I_{PTX} remaining after MTSET⁺ application in the experiments of Fig. 2e, right, was $57 \pm 2\%$ ($n = 3$) when measured at -150 mV; this good correlation between macroscopic and microscopic data is expected given the high open probability (~ 0.9) of G805C (**c**) and T806C (**d**) pump-channels. All data are the mean $\pm$ s.e.m.

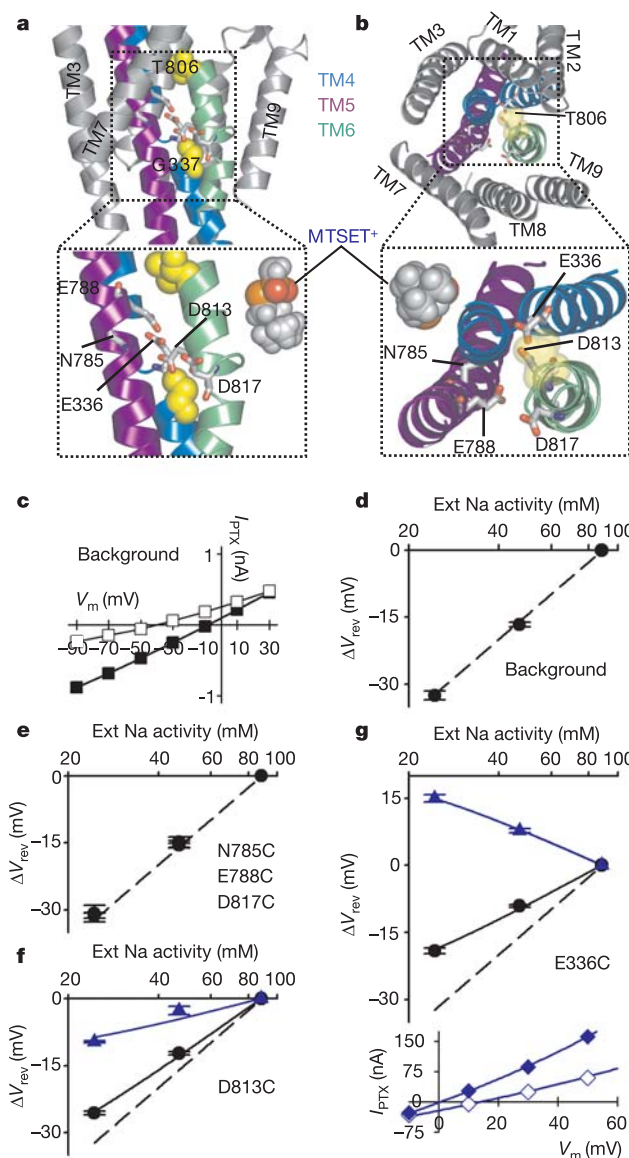


Figure 4 | Charge selectivity in palytoxin-bound pump-channels. **a, b**, Na⁺, K⁺ pump homology model (Fig. 1) viewed parallel to the membrane plane (**a**) or from the extracellular surface (**b**), with MTSET⁺ shown for size reference. **c**, Pump-channels retaining all indicated native residues (Background) showed a large negative shift of V_{rev} on decreasing external NaNO₃ concentration (filled squares, 120 mM; open squares, 30 mM). **d–g**, Semi-log plots of ΔV_{rev} versus external Na⁺ activity. Results from background pump-channels (**d**) and from N785C, E788C or D817C mutants (**e**) approximate the broken line, which is appropriate to exclusive cation selectivity and calculated as $\Delta E_{\text{rev}} = (RT/F)\ln(X)$, where X is the ratio of the lower to the higher monovalent cation activity. Mutations D813C (**f**) and E336C (**g**) each weakened cation-to-anion selectivity (black circles and fit lines). MTSET⁺ treatment (blue triangles and fit lines) further impaired the cation selectivity of D813C channels (**f**) and rendered E336C channels (**g**) anion selective; inset in **g** shows the positive ΔV_{rev} due to a fourfold decrease in external NaNO₃ concentration (filled to open blue diamonds) in MTSET⁺-modified E336C channels. Residues corresponding to E336, N785, E788, D813 and D817 in the Ca²⁺-ATPase are E309, N768, E771, N796 and D800. Error bars indicate the s.e.m.

to the strong cation selectivity of palytoxin-bound pump-channels, which is impaired when either negative charge is attenuated (by mutation to cysteine) and is further compromised (position 813) or switched to anion selectivity (position 336) when a positive charge is added (by reaction with MTSET⁺).

Our results show that extracellular ions enter Na⁺,K⁺ pump-channels through a wide vestibule that extends to the hydrophobic core of the membrane before suddenly narrowing to form a cation-selective pore. The principal cation-selectivity determinants are acidic residues expected to form binding sites in which Na⁺ and K⁺ ions are normally occluded. Evidence suggests that this architecture is conserved in the related H⁺,K⁺- and Ca²⁺-ATPases, and in Na⁺,K⁺-ATPase without palytoxin. An external cavity observed in electron microscopic images of thapsigargin-stabilized E2-like Ca²⁺-ATPase¹⁹ has been confirmed in crystal structures of E2P-related Ca²⁺-ATPase forms (E2·AlF₄⁻, ref. 20; E2·MgF₄²⁻, ref. 10). E2P forms of Na⁺,K⁺ and H⁺,K⁺ pumps also apparently include an external cavity large enough to accommodate bulky extracellular inhibitors such as the cardiotonic steroid ouabain (Na⁺,K⁺ pump) or omeprazole (H⁺,K⁺ pump): both bind to E2P conformations^{6,21,22}; and high-affinity binding of ouabain requires T806 of Na⁺,K⁺-ATPase (for example, see ref. 21), whereas omeprazole covalently binds to the T806-equivalent residue C813 of the H⁺,K⁺ pump (for example, see refs 6, 22).

The fact that a narrow access channel connects ion-binding sites in the Na⁺,K⁺ pump to the extracellular solution has been inferred from the membrane-potential dependence of extracellular Na⁺ and K⁺ action (for example, see ref. 23). In addition, a narrow fissure in the E2·MgF₄²⁻ structure, penetrating from the T806-equivalent residue (P789) of the Ca²⁺-ATPase towards Ca²⁺-binding site II, has been proposed as a possible exit route for released Ca²⁺ (ref. 10; but see also refs 11, 12). At binding site II, between TM4 and TM6 in the E1·2Ca²⁺ structure⁷, side-chain oxygen atoms of residues equivalent to E336 (E309) and D813 (N796) coordinate Ca²⁺. E336 and D813 are therefore modelled as components of the corresponding Na⁺,K⁺ pump site, alternately coordinating Na⁺ ions in E1 and a K⁺ ion in E2 conformations^{5,9}, and their key roles in both Na⁺,K⁺- and Ca²⁺-ATPases are corroborated by the functional consequences of mutating them^{16,24,25}. We found that palytoxin-bound pump-channels with cysteine substitutions at N785, E788 or D817—also modelled to coordinate occluded Na⁺ and K⁺ ions⁵—remained highly cation selective and unresponsive to extracellular 1 mM MTSET⁺. This might reflect rearrangement of the coordinating sites when the ion pathway opens. Binding-site structure indeed differs between the Ca²⁺-bound⁷ and unbound deoccluded^{10,11} Ca²⁺-ATPase states. In the latter only side chains of the E336- and D813-equivalent residues occupy the space between TM4 and TM6 (compare Fig. 4b and Supplementary Figs 3 and 4), whereas in the former the D817-equivalent side chain also enters this space (Supplementary Fig. 4). Our results imply that in an open E2P-like state, with ion-binding sites accessible from the extracytoplasmic side of the membrane, the arrangement of coordinating residues resembles that in the deoccluded E2P-related structures^{10,11,20}.

We conclude that, in the E2P conformation of the Na⁺,K⁺ pump, Na⁺ and K⁺ exchange between site II and the external medium occurs through a funnel-shaped pathway, which is lined in part by TM4 and TM6 and comprises a cation-selectivity filter formed by acidic residues in the ion-coordination site. We propose that the extracytoplasmic ion exchange pathways of Ca²⁺-, H⁺,K⁺- and Na⁺,K⁺-ATPase cation pumps share these physical characteristics. These methods should facilitate further characterization of the ion translocation pathway of the Na⁺,K⁺ pump, including the locations of the gates that control access to the ion-binding sites.

METHODS

Model building. The *Xenopus* Na⁺,K⁺-ATPase α_1 subunit homology model was built from the Ca²⁺-ATPase E2·MgF₄²⁻ structure¹⁰ (PDB code 1WPG) with

SWISS-MODEL (<http://swissmodel.expasy.org>). Structural figures were prepared with PyMOL version 0.97 (<http://www.pymol.org>).

Ouabain- and MTS-insensitive Na⁺,K⁺ pumps. Na⁺,K⁺ pumps insensitive to ouabain²⁶ and MTS reagents¹⁴ were made by introducing the point mutations Q120D, N131R and C113S into *Xenopus* α_1 subunits²⁷. All other mutations were introduced into these Q120D-N131R-C113S Na⁺,K⁺ pumps, which generate robust extracellular-K⁺-activated currents in Na⁺-loaded oocytes (data not shown). Complementary RNAs of mutant *Xenopus* α_1 catalytic and wild-type *Xenopus* β_3 auxiliary subunits²⁸ were coinjected in a 2:1 ratio into *Xenopus* oocytes. The mutation N131R decreased the conductance of single palytoxin-bound pump-channels to ~1.5 pS, as compared with ~4 pS for the wild-type (N131) channel²⁹.

Current recordings. Macroscopic or microscopic currents were recorded at 22–24°C in outside-out patches excised from *Xenopus* oocytes, 1–3 d after injection. Internal (pipette) solutions contained (in mM): 120 NaOH, 90 sulphamic acid, 1 MgCl₂, 10 HEPES and 10 EGTA. External solutions contained (in mM): 120 NaOH or *N*-methyl-D-glucamine (NMDG), 133 sulphamic acid, 5 Ba(OH)₂, 0.5 Ca(OH)₂, 1 Mg(OH)₂ and 10 HEPES. Palytoxin was added to external 120 mM Na⁺ solutions at concentrations of 100 nM for macroscopic, or 100 pM for microscopic, recording. All external solutions contained 100 μ M ouabain to preclude palytoxin-induced responses from endogenous pumps.

Cation/anion selectivity. For experiments varying Na⁺ activity, sulphamic acid was replaced by nitric acid and sucrose was added to maintain osmolality (250 $\pm$ 10 mosmol kg⁻¹). P_{Na}/P_{NO_3} ratios were obtained by fitting the Goldman-Hodgkin-Katz equation, $V_{rev} = RT/F \cdot \ln\{(P_{Na}/P_{NO_3})[Na^+]_o + [NO_3^-]_i\} / (P_{Na}/P_{NO_3}[Na^+]_i + [NO_3^-]_o)$, where the subscripts o and i indicate external and internal ion activities³⁰, respectively, to values of ΔV_{rev} as a function of Na⁺ and NO₃⁻ activity. All data are reported as the mean $\pm$ s.e.m.

Received 31 May; accepted 26 July 2006.

1. Lauger, P. *Electrogenic Ion Pumps* (Sinauer, Sunderland, Massachusetts, 1991).
2. Artigas, P. & Gadsby, D. C. Na⁺/K⁺-pump ligands modulate gating of palytoxin-induced ion channels. *Proc. Natl Acad. Sci. USA* **21**, 501–505 (2003).
3. Karlin, A. & Akabas, M. H. Substituted-cysteine accessibility method. *Methods Enzymol.* **293**, 123–145 (1998).
4. Sweadner, K. J. & Donnet, C. Structural similarities of Na,K-ATPase and SERCA, the Ca²⁺-ATPase of the sarcoplasmic reticulum. *Biochem. J.* **356**, 685–704 (2001).
5. Ogawa, H. & Toyoshima, C. Homology modeling of the cation binding sites of Na⁺/K⁺-ATPase. *Proc. Natl Acad. Sci. USA* **99**, 15977–15982 (2002).
6. Munson, K., Garcia, R. & Sachs, G. Inhibitor and ion binding sites on the gastric H,K-ATPase. *Biochemistry* **44**, 5267–5284 (2005).
7. Toyoshima, C., Nakasako, M., Nomura, H. & Ogawa, H. Crystal structure of the calcium pump of sarcoplasmic reticulum at 2.6 Å resolution. *Nature* **405**, 647–655 (2000).
8. Toyoshima, C. & Nomura, H. Structural changes in the calcium pump accompanying the dissociation of calcium. *Nature* **418**, 605–611 (2002).
9. Rakowski, R. F. & Sagar, S. Found: Na⁺ and K⁺ binding sites of the sodium pump. *News Physiol. Sci.* **18**, 164–168 (2003).
10. Toyoshima, C., Nomura, H. & Tsuda, T. Luminal gating mechanism revealed in calcium pump crystal structures with phosphate analogues. *Nature* **432**, 361–368 (2004).
11. Moller, J. V., Nissen, P., Sorensen, T. L.-M. & le Marie, M. Transport mechanism of the sarcoplasmic reticulum Ca²⁺-ATPase pump. *Curr. Opin. Struct. Biol.* **15**, 387–393 (2005).
12. Picard, M., Toyoshima, C. & Champeil, P. Effects of inhibitors on luminal opening of Ca²⁺ binding sites in an E2P-like complex of sarcoplasmic reticulum Ca²⁺-ATPase with Be²⁺-fluoride. *J. Biol. Chem.* **281**, 3360–3369 (2006).
13. Scheiner-Bobis, G., Meyer zu Heringdorf, D., Christ, M. & Habermann, E. Palytoxin induces K⁺ efflux from yeast cells expressing the mammalian sodium pump. *Mol. Pharmacol.* **45**, 1132–1136 (1994).
14. Guennoun, S. & Horisberger, J.-D. Structure of the 5th transmembrane segment of the Na,K-ATPase α subunit: a cysteine-scanning mutagenesis study. *FEBS Lett.* **482**, 144–148 (2000).
15. Horisberger, J.-D. Recent insights into the structure and mechanism of the sodium pump. *Physiology (Bethesda)* **19**, 377–387 (2004).
16. Nielsen, J. M., Pedersen, P. A., Karlsh, S. J. & Jorgensen, P. L. Importance of intramembrane carboxylic acids for occlusion of K⁺ ions at equilibrium in renal Na,K-ATPase. *Biochemistry* **37**, 1961–1968 (1998).
17. Artigas, P. & Gadsby, D. C. Large diameter of palytoxin-induced Na/K pump channels and modulation of palytoxin interaction by Na/K pump ligands. *J. Gen. Physiol.* **123**, 357–376 (2004).
18. Keramidas, A., Moorhouse, A. J., Schofield, P. R. & Barry, P. H. Ligand-gated ion channels: mechanisms underlying ion selectivity. *Prog. Biophys. Mol. Biol.* **86**, 161–204 (2004).
19. Zhang, P., Toyoshima, C., Yonekura, K., Green, N. M. & Stokes, D. L. Structure of the calcium pump from sarcoplasmic reticulum at 8-Å resolution. *Nature* **392**, 835–839 (1998).

20. Olesen, C., Sorensen, T. L., Nielsen, R. C., Moller, J. V. & Nissen, P. Dephosphorylation of the calcium pump coupled to counterion occlusion. *Science* **306**, 2251–2255 (2004).
21. Qiu, L. Y., Koenderink, J. B., Swarts, H. G., Willems, P. H. & De Pont, J. J. Phe⁷⁸³, Thr⁷⁹⁷, and Asp⁸⁰⁴ in transmembrane hairpin M5–M6 of Na⁺,K⁺-ATPase play a key role in ouabain binding. *J. Biol. Chem.* **278**, 47240–47244 (2003).
22. Asano, S. *et al.* The cavity structure for docking the K⁺-competitive inhibitors in the gastric proton pump. *J. Biol. Chem.* **279**, 13968–13975 (2004).
23. Apell, H. J. Structure–function relationship in P-type ATPases—a biophysical approach. *Rev. Physiol. Biochem. Pharmacol.* **150**, 1–35 (2003).
24. Vilsen, B. & Andersen, J. P. Mutation to the glutamate in the fourth membrane segment of Na⁺,K⁺-ATPase and Ca²⁺-ATPase affects cation binding from both sides of the membrane and destabilizes the occluded enzyme forms. *Biochemistry* **37**, 10961–10971 (1998).
25. Inesi, G., Ma, H., Lewis, D. & Xu, C. Ca²⁺ occlusion and gating function of Glu³⁰⁹ in the ADP-fluoroaluminate analog of the Ca²⁺-ATPase phosphoenzyme intermediate. *J. Biol. Chem.* **279**, 31629–31637 (2004).
26. Price, E. M., Rice, D. A. & Lingrel, J. B. Structure–function studies of Na,K-ATPase: site-directed mutagenesis of the border residues from the H1–H2 extracellular domain of the α subunit. *J. Biol. Chem.* **265**, 6638–6641 (1990).
27. Verrey, F. *et al.* Primary sequence of *Xenopus laevis* Na⁺-K⁺-ATPase and its localization in A6 kidney cells. *Am. J. Physiol.* **256**, F1034–F1043 (1989).
28. Good, P. J., Richter, K. & Dawid, I. B. A nervous system-specific isotype of the β subunit of Na⁺,K⁺-ATPase expressed during early development of *Xenopus laevis*. *Proc. Natl Acad. Sci. USA* **87**, 9088–9092 (1990).
29. Artigas, P. & Gadsby, D. C. Ouabain affinity determining residues lie close to the Na/K pump ion pathway. *Proc. Natl Acad. Sci. USA* **103**, 12613–12618 (2006).
30. Grenthe, I., Plyasunov, A. V. & Spahiu, K. In *Modelling in Aquatic Chemistry* (eds Grenthe, I. & Puigdomenech, I.) 325–426 (Organisation for Economic Co-operation and Development (OECD) Publications, Paris, 1997).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank P. Artigas for advice and discussion; M. Mense and P. Vergani for discussion; and R.F. Rakowski for cDNAs encoding the *Xenopus* α_1 and β Na⁺,K⁺-ATPase subunits. This work was supported by a grant from the NIH (to D.C.G.).

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to D.C.G. (gadsby@rockefeller.edu).

naturejobs

**THE CAREERS
MAGAZINE FOR
SCIENTISTS**

Five years on, an experiment to reform career paths for German researchers has posted mixed results. The plan began with good intentions: to get promising young professors into permanent positions more quickly. To do this, the federal government created the new title of 'junior professor', the idea being that these positions would give young researchers more autonomy earlier in their career.

But some *Länder*, or states, in Germany rejected the idea, leaving the country with a mixed system. The more politically liberal *Länder* opted to include some of the new positions in their universities, whereas the more conservative *Länder* eschewed them. Germany's highest court said that the federal government couldn't intrude on the way individual *Länder* structured academic advancement.

Then the more conservative *Länder* challenged changes to the traditional German process of obtaining tenure known as *Habilitation*, which mandates a second dissertation. With *Habilitation* still intact, the path towards independence becomes even more confusing. Is a junior professor less competitive than a colleague who takes *Habilitation*? If so, should a young scientist who takes a junior professorship still do the *Habilitation* dissertation in order to remain competitive with colleagues who pursue the traditional path? If that's the case, then what's the advantage of accepting a junior professorship?

Junior professors who don't do a *Habilitation* and young researchers who do the second dissertation have one thing in common — neither can pursue jobs at the university where they did their training. This is different from the situation in the United States, where a junior professor can remain as a full professor once tenure has been granted.

For jobseekers looking to Germany, this muddled scenario should prompt them to ask several questions before they take on a position. Or, if they can't get their heads around the system, they may do what many of their colleagues have been doing — pursue a professorship in a different country.

Paul Smaglik, *Naturejobs* editor

Publisher: Ben Crowe
Editor: Paul Smaglik
Assistant Editor: Gene Russo

European Head Office, London
The Macmillan Building,
4 Crinan Street, London N1 9XW, UK
Tel: +44 (0) 20 7843 4961
Fax: +44 (0) 20 7843 4996
e-mail: naturejobs@nature.com

European Sales Manager:
Andy Douglas (4975)
e-mail: a.douglas@nature.com
Business Development Manager:
Amelie Pequignot (4974)
e-mail: a.pequignot@nature.com
Natureevents:
Claudia Paulsen Young (+44 (0) 20 7014 4015)
e-mail: c.paulsenyoung@nature.com
France/Switzerland/Belgium:
Muriel Lestranguez (4994)

UK/Ireland/Italy/RoW:
Loredana Milanese (4944)
Nils Moeller (4953)
Scandinavia/Spain/Portugal:
Evelina Rubio-Morgan (4973)
Germany/Austria/The Netherlands:
Reya Silao (4970)
Online Job Postings:
Matthew Ward (+44 (0) 20 7014 4059)

European Satellite Office
Germany: Patrick Phelan
Tel: +49 89 54 90 57 11
Fax: +49 89 54 90 57 20
e-mail: p.phelan@nature.com

Advertising Production Manager:
Stephen Russell
To send materials use London address above.
Tel: +44 (0) 20 7843 4816
Fax: +44 (0) 20 7843 4996
e-mail: naturejobs@nature.com

Naturejobs web development: Tom Hancock
Naturejobs online production:
Catherine Alexander

US Head Office, New York
75 Varick Street, 9th Floor,
New York, NY 10013-1917
Tel: +1 800 989 7718
Fax: +1 800 989 7103
e-mail: naturejobs@natureny.com

US Sales Manager: Peter Bless

Japan Head Office, Tokyo
Chiyoda Building, 2-37 Ichigayatamachi,
Shinjuku-ku, Tokyo 162-0843
Tel: +81 3 3267 8751
Fax: +81 3 3267 8746

Asia-Pacific Sales Manager:
Ayako Watanabe
e-mail: a.watanabe@natureasia.com



CORBIS

Building a better foundation

Born of extraordinary fortunes, research-funding foundations are often efforts to keep a family or individual name alive through philanthropy. Eccentric American anglophile Henry Wellcome willed millions to start the Wellcome Trust, Europe's largest foundation dedicated to research. Similarly, Istanbul-born Calouste Gulbenkian left money to fund the sciences and arts in Portugal, where he died in 1955.

Europe could be doing more to preserve and enhance such legacies, but policies and tradition seem to stand in the way. There is an expectation in Europe that the state should fund research and education, for example. And some countries in the European Union (EU) lack the tax-break incentives that would encourage giving. In addition, compared with in the United States, there are fewer super-rich people in Europe anxious to donate part of their fortune to an institution. Gulbenkian and Wellcome are two of the few European foundations that have a significant impact on their host countries' research and development (R&D).

Yves Mény, president of the European University Institute in Florence, Italy, wants this trend to change. Two years ago, he told the European Commission (EC) that foundations in Europe — broadly speaking, non-profit organizations whose income is derived from a large endowment and/or charitable giving — can and should do more. The commission took notice. Officials asked Mény to convene an expert panel of foundation representatives from across Europe to explore how to boost the role of philanthropy in European R&D.

"The purpose of the report was to say we lack a legal framework and a financial framework that could contribute to incentives for foundations to operate European-wide and not only at a national or local level," Mény says.

The deliberations of the panel — which included

Europeans have traditionally expected their governments to fund research. But foundations are hoping to play a major new role, says **Gene Russo**.

representatives from Germany's Volkswagen Foundation, the Gulbenkian Foundation, the Wellcome Trust and an umbrella organization called the European Foundation Centre — culminated in a report released early this year. A meeting of numerous other foundation representatives followed in March. It was the first time the EC had ever convened an event to discuss the role of foundations, trusts and charities in supporting research in Europe, according to speaker Janez Potočnik, commissioner for research at the EC. The rising level of interest signals a "sea change" in the EU's relationship with the non-profit sector, says panel participant and senior solicitor at the Wellcome Trust, Eleanor Boddington.

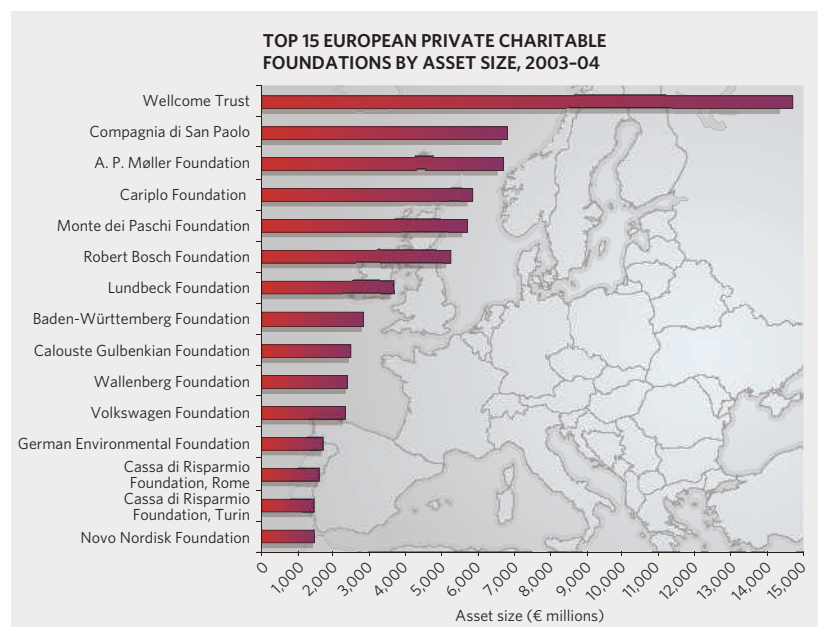
Changing role

The European Council meeting in March 2002 agreed to try to increase investment in research and development from 2% to 3% of total GDP by 2010, with at least two-thirds coming from the private sector. But Mény and other researchers have serious doubts about the EC's bureaucratic Seventh Framework funding programme, which requires that scientists, in order to earn grants, form research collaborations with partners from multiple countries. Sometimes these networks are awkward, forced and inefficient, say critics. Research funded by the newly proposed European Research Council, part of the framework, will have more flexibility. But, Mény argues, the research council is likely to be made inefficient and sluggish by an avalanche of applications. "It might be a bureaucratic monster," he says.

Instead, Mény and his foundation colleagues envisage a distributed system of funding in which the EC matches funds put up by foundations and universities. The commission could prioritize research areas, but the projects would be the responsibility of the institutions, provided they act according to basic rules

Yves Mény is keen to see foundations play a bigger role in European R&D.





that govern, for example, grantee selection and assessment. It's a more efficient system, Mény argues. Right now, it's a pipedream. But Mény and the other meeting participants believe that foundations can, at least, magnify their impact and spark innovation.

Working in concert

Can European foundations really make a difference? Although governments have far more resources, foundations have some advantages. Usually, they are quicker to act and more likely to take chances on riskier, less proven projects. They're also not beholden to taxpayers, political consensus or shareholders.

In some cases, foundations serve as catalysts for a national programme. Britain is the most obvious example. Together the Wellcome Trust and Cancer Research UK, a charity that relies largely on donations, devote more money to biomedical R&D than the UK government's Medical Research Council. With an endowment of some £12,300 million (US\$23,300 million), Wellcome's assets are by far the largest of the European foundations (see graph). The trust, which spends about £450 million a year on research has jointly funded several projects with the government. In 1998, it devoted £300 million to the £750-million Joint Infrastructure Fund to improve researchers' facilities and equipment, and devoted more than £50 million towards the construction of a third-generation synchrotron radiation source for structural and molecular biology studies.

António Coutinho, director of the Gulbenkian Science Institute, says that the foundation has helped bring modern biology to Portugal, supporting the study of single nucleotide polymorphisms, gene-expression techniques and bioinformatics. The government has now become a bigger contributor. In the 1960s, Gulbenkian funded about 40% of Portuguese research, according to Coutinho; now it funds closer to 1%.

The French Muscular Dystrophy Association (AFM) started in 1958, but didn't blossom until it staged an all-day telethon on national television in 1987 to raise funds for research into neuromuscular diseases. The telethon now brings in €100 million (US\$126 million) a year, two-thirds of which goes to research. Although

this is small compared with French government support, the AFM has contributed to major initiatives. A very preliminary mapping of the human genome that it funded was used by scientists working on the Human Genome Project in the early 1990s.

The telethon model has now spread to Italy, Belgium, Luxembourg and Spain, and efforts are under way to establish an association in Germany. Italian researcher and telethon grantee Giulio Cossu, director of the Stem Cell Research Institute in Milan, says that the foundation has helped him work on a rare disease that usually garners few funding opportunities. It also helped him find an alternative to an Italian government R&D funding regime well-known for bureaucracy and favouritism. Italian telethon scientific director Francesca Pasinelli says they get many more requests for large projects that their €33-million yearly budget can meet.

Increasing visibility

The report drawn up earlier this year cites several steps that European foundations could take. Panel members called for European foundations to increase their visibility and better document their Europe-wide contributions to research. Universities, the panel recommended, should create their own foundations to generate funds and attract alumni donations. And a European Foundation Statute should be created to let foundations based in one EU country operate easily in others; unlike businesses, foundations have to register in every country in which they plan to operate. The panel also called for a European Forum of Research Foundations, an initiative now being organized by the European Foundation Centre. The forum would meet regularly and discuss best practices and ways to create synergies among foundations.

One of the trickier recommendations is EU-wide improved and standardized tax credits for foundations and potential donors. Individual countries are starting to recognize the benefits of tax breaks. This year, for the first time, says Cossu, Italian citizens can donate up to 5% of their tax payment to non-profit organizations that fund research. In Norway, where philanthropic support for research has never been encouraged, a new scheme could help to spark giving. The government will match up to 25% of the funds that a company or person donates to research. The move has attracted some interest from other EU countries, according to panel member Tore Li, a senior adviser with the Confederation of Norwegian Enterprise.

Devising a pan-European tax policy could help as well. A German citizen who wants to donate to a French foundation, for example, won't get a tax break. But getting EU countries to agree on reciprocal tax rules will be difficult, Mény concedes. "We know that with the European experience, we have to go step by step, we have to talk a lot about the issues," he says.

Next up will be an EC-sponsored meeting, likely to be held next year, about the role of European universities, and how they can use funds from philanthropic sources. It's part of what proponents hope is a movement towards better coordination among foundations, better incentives for donors and founders, and a greater awareness among Europeans that foundations can push the frontiers of innovation. Only then might the desire to leave a lasting philanthropic legacy help Europe reach its R&D potential.

Gene Russo is assistant editor of *Naturejobs*.

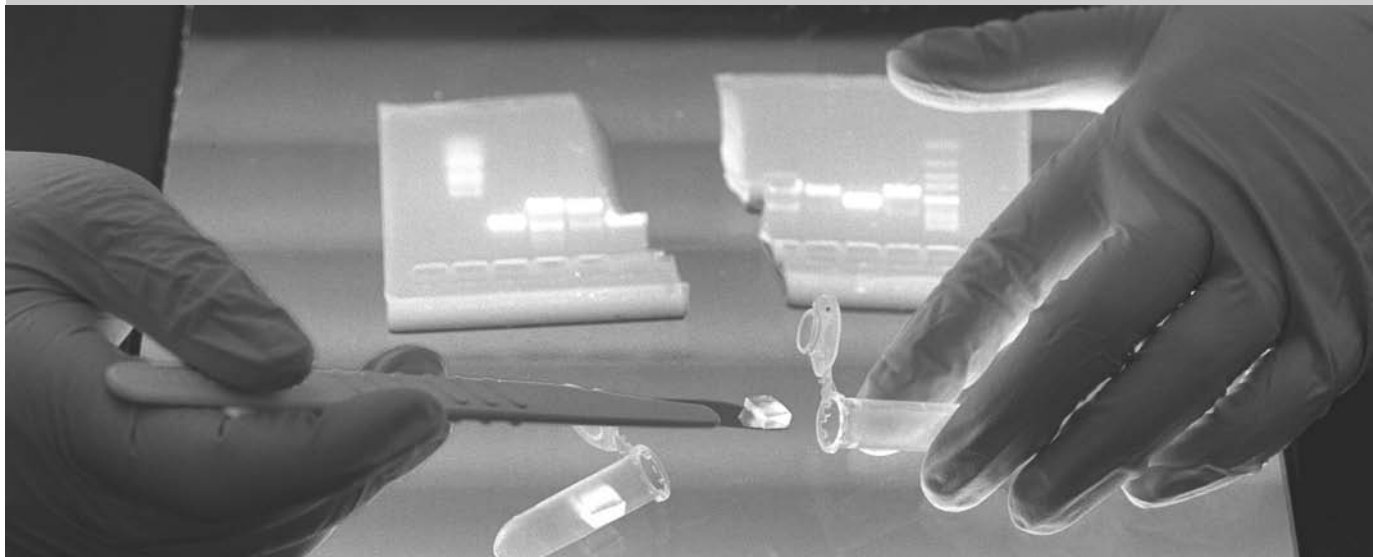
Giulio Cossu says foundation money has made his research possible.



Forschungszentrum Jülich
in der Helmholtz-Gemeinschaft



Wir sind das größte interdisziplinäre Forschungszentrum in Europa und arbeiten auf den Gebieten „Energie“, „Information“, „Leben“ und „Umwelt“.



So gestalten wir Zukunft:

Wir erforschen

- umweltfreundliche Energiesysteme der Zukunft
- Informationstechnik der übernächsten Generation
- Gesundheit und lebenswichtige Substanzen für den Menschen sowie
- die Basis für eine lebenswerte Umwelt

Junge Menschen – unsere Zukunft

Deshalb

- fördern wir das Interesse der Jugend an den Naturwissenschaften
- bilden wir junge Menschen in ca. 20 Berufen aus
- bieten wir Diplomarbeiten
- arbeiten bei uns zahlreiche junge Wissenschaftlerinnen und Wissenschaftler an ihrer Dissertation

Unser Frauenförderungs-Programm gilt als „Maßstab in der deutschen Forschungslandschaft“.

Wir fördern

- klare Rahmenbedingungen für Chancengleichheit
- Vereinbarkeit von Familie und Beruf
- Frauen in Führungspositionen

Chancengleichheit ist Bestandteil unserer Personalpolitik. Bewerbungen Schwerbehinderter sind uns willkommen.

Möchten Sie mehr wissen? Schreiben Sie uns:
Forschungszentrum Jülich GmbH, Geschäftsbereich Personal – Personalentwicklung, Wilhelm-Johnen-Straße, 52425 Jülich.
Rufen Sie an: 02461 61-5358, Fax: 02461 61-8035.

Oder besuchen Sie uns im Internet: <http://www.fz-juelich.de>.
Dort finden Sie auch interessante Stellenangebote.

W89258R



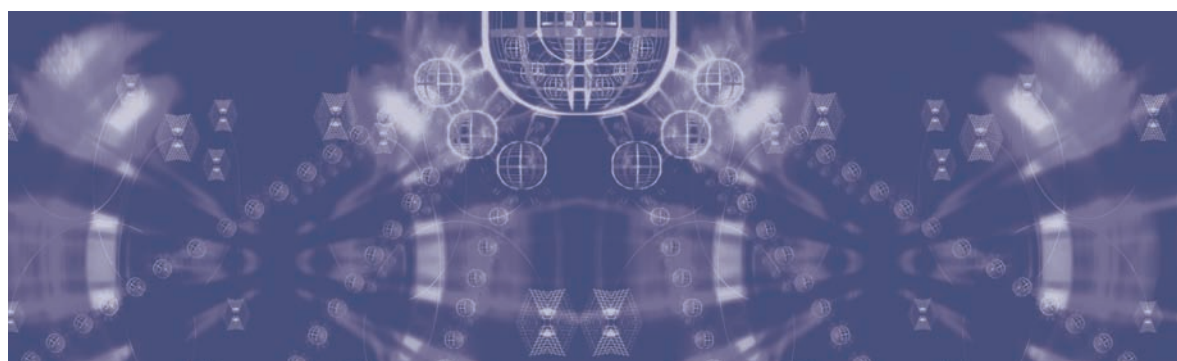
HELMHOLTZ
| GEMEINSCHAFT

www.helmholtz.de

SHAPING THE FUTURE THROUGH SCIENTIFIC EXCELLENCE

The Helmholtz Association of German Research Centres is an alliance of 15 autonomous science facilities dedicated to the pursuit of long-term strategic research goals. The Association and institutes have a total staff of 25,000 and an annual budget of more than 2.2 billion euros.

Helmholtz scientists work in the research fields of Energy, Earth and Environment, Health, Key Technologies, Structure of Matter and Transport and Space and use the most modern scientific infrastructure, in particular, large-scale facilities and instrumentation. Helmholtz scientists pursue an ambitious goal: To make a significant contribution to solving the grand challenges facing society.



PROMOTING YOUNG SCIENTISTS AND RESEARCHERS: Advancing Germany as a centre for top-class research – that is one of the Helmholtz Association's key objectives. To this end the Association is intensifying its cooperation with partners from business, industry and research, concentrating expertise across disciplinary boundaries. We place particular importance on promoting young scientists and researchers.

Post-doctorate students from Germany and abroad can apply for positions as Helmholtz Young Investigator Group Leaders. They are given the opportunity to build their own groups and perform independent research under optimal working conditions, with reliable career prospects including a tenure option. The young scientists work closely with a university and can in this way gain valuable teaching experience. The Helmholtz Association presently trains about 3,000 doctoral students in coop-

eration with universities. Excellent scientific training is of particular importance and the young researchers are given intensive personal support. Up to 25 particularly well-qualified doctoral students are enrolled in each of the Helmholtz Research Schools, which offer a range of transferable scientific skills. In addition, Helmholtz Graduate Schools are planned, which will provide doctoral students from the Helmholtz Centres with high-level training on a wider scale.

Alfred Wegener Institute (AWI)

Deutsches Elektronen-Synchrotron (DESY)

German Cancer Research Centre (DKFZ)

German Aerospace Centre (DLR)

Research Centre Jülich

Forschungszentrum Karlsruhe

GeoForschungsZentrum Potsdam (GFZ)

GKSS Forschungszentrum Geesthacht

GSF-National Research Centre
for Environment and Health

Gesellschaft für Schwerionenforschung (GSI)

Hahn-Meitner Institute (HMI)

Helmholtz Centre for Infection Research

Max-Planck-Institut für Plasmaphysik (IPP)

Max Delbrück Centre for
Molecular Medicine (MDC) Berlin-Buch

UFZ Centre for Environmental Research Leipzig-Halle

Deutsches Elektronen-Synchrotron FEL-Research

DESY is world-wide one of the leading accelerator centres exploring the structure of matter. The main research areas range from elementary particle physics over various applications of synchrotron radiation to the construction and use of X-ray lasers.

The Free-Electron-Laser FLASH at DESY provides unique opportunities for research using shortwavelength radiation. For soft X-ray pump-probe experiments we are seeking to recruit a

Postdoc (Ph.D.) # 99/2006

for experiments investigating dense plasma states created by an intense optical fs-laser in two-colour pump-probe experiments. An upgrade of the existing OPCPA fs-laser system is planned to achieve even higher intensities for the optical laser. You will perform pump-probe experiments within an international collaboration and will lead the laser upgrade.

Young scientists who have completed their Ph.D. are invited to submit their application including a resume and the usual documents (curriculum vitae, list of publications and copies of university degree). Candidates should have a solid background in ultrashort pulsed laser physics and/or plasma physics. Ability to work in a team and experience in the coordination of small projects are advantageous. If you are interested in this position you may contact T. Tschentscher (thomas.tschentscher@desy.de) or S. Düsterer (stefan.duesterer@desy.de) for further information. Please send your application papers to our personnel department.

The position is limited to 2 years with the possibility to extend by 1 year.

Salary and benefits are commensurate with public service organisations. DESY is an equal opportunity, affirmative action employer and encourages applications from women. DESY has a Kindergarten on site.

Closing date: October 31, 2006

Particle accelerators produce high intensive radiation for most diverse innovative applications. By the forthcoming upgrade of the PETRA storage ring into a 3rd generation synchrotron light source, the operation of the free-electron laser FLASH and the construction of the European XFEL DESY will take up an international top position in research with photons. Amongst others X-ray free-electron laser research opens a new area of time-resolved and coherent X-ray scattering applications. For the conception, planning and realization of FEL experiments in the field of soft X-ray imaging we are seeking to recruit a

Scientist (Ph.D.) # 100/2006

The successful candidate will prepare and carry out experiments at the FLASH facility at DESY in Hamburg and at the Linac Coherent Light Source (LCLS) at SLAC/Stanford, USA. This work will be an important precursor for experiments at the future European XFEL facility in Hamburg. Candidates should hold a Ph.D. in physics or in a related field and have a proven record in synchrotron radiation research or in modern (short pulse) laser physics. Experience with soft X-ray radiation, time-resolved or coherence X-ray experiments will be of advantage. If you are interested in this position, please send your complete application by indicating the reference number to our personnel department.

For further information you can contact T. Tschentscher (thomas.tschentscher@desy.de) or G. Grübel (gerhard.gruebel@desy.de).

Salary and benefits are commensurate with public service organisations. DESY is an equal opportunity, affirmative action employer and encourages applications from women. DESY has a Kindergarten on site.

Closing date: October 31, 2006

Deutsches Elektronen-Synchrotron DESY member of the Helmholtz Association

**Notkestraße 85 · 22603 Hamburg · Germany
Phone: 040/89983392 · www.desy.de
email: personal.abteilung@desy.de**

Centre for Environmental Research Leipzig-Halle in der Helmholtz-Gemeinschaft

Research for the Environment

As an international centre responsible for environmental research, the UFZ is investigating the interaction between man and environment in used and disturbed landscapes. The UFZ develops concepts and methods to ensure a healthy environment for future generations.

The Department of Soil Ecology has openings for

2 Senior Scientists (m/f) in the field of Soil Micro-Organisms/ Mycorrhizal Symbioses

**with one position in Biodiversity and
one position in Molecular Genetic**

The latest starting date for both positions is 01.12.2006.

The candidates will work directly under and report to the head of the department that supervises current projects, employing 1 post-doc, 4-7 PhD students and 1-3 technicians. The department is also tasked with preparing new projects either within the Helmholtz Association or funded by external, national and international resources.

The position in Biodiversity combines tools in the fields of cytology, image analysis and molecular ecology for tracing the diversity of soil fungi and of ecto- and arbuscular mycorrhizas in both field studies and experiments in Europe and the tropics. The position in Molecular Genetics will involve the development of genomic, transcriptomic and proteomic tools for high throughput studies of the structure and function of soil micro-organisms and mycorrhizas in their environment and in culture systems.

Requirements: Both applicants should have a Ph.D. in Biology, Microbiology, Molecular Ecology or Bioinformatics, as well as international post-doc. experience and competence in project management. They should be fluent in English (German appreciated), have an in-depth knowledge of statistics and be able to cooperate with modellers. The candidate for the position in Biodiversity must have extensive experience in biology, ecology and the molecular phylogeny of fungi and mycorrhizas. The candidate for the position in molecular genetics must be able to master the tools for constructing and screening (meta-)genomic banks, monitoring gene expression and protein synthesis, including transformation systems, as well as developing and using micro-arrays.

Terms and conditions: Both positions supported by the UFZ are for a period of 2 years initially.

For further information, please contact Prof. Dr. François Buscot, T +49 (0)345-558-5221, francois.buscot@ufz.de (see also www.ufz.de).

Salaries will be in accordance with the appropriate German civil service level (TVöD-O).

Women are explicitly encouraged to apply, as we wish to increase their share in science and research. Physically-handicapped persons will be favoured if they are equally qualified.

Contact: Applicants should send their C.V., including a list of publications, a short summary of research interests, and the names (and e-mail address) of at least two references to the Personnel Department of the **UFZ-Centre for Environmental Research Permoserstr. 15, 04318 Leipzig, Germany** quoting reference number 63/2006 for the position in Biodiversity and for the position on Molecular Genetic.

Closing date: October 15, 2006

Forschungszentrum Karlsruhe GmbH in der Helmholtz-Gemeinschaft

The Forschungszentrum Karlsruhe GmbH, a member of the Helmholtz Association, is one of the leading research centres in Europe. Its Institute for Synchrotron Radiation operates the synchrotron light source known as ANKA. This is a national user facility that provides light from hard X-rays to the far-infrared for spectroscopy, scattering, imaging and lithography in research and technology. Special attention is given to the industrial use of ANKA by the independent unit ANKA Commercial Service (ANKA CoS).

Objectives: ANKA focuses on the use of synchrotron radiation techniques for micro- and nano technologies, condensed matter research, actinide and environmental research in addition to the development of synchrotron technology. ANKA CoS provides a fast and flexible service for industrial customers and is continually developing products and applications for new markets.

Requirements: As part of the current expansion of the ANKA project, we are looking for the following staff to strengthen our team:

Senior scientists (f/m) Scientists (f/m)

Project Managers – commercial service (f/m) PhD students (f/m)

Detailed information about these vacancies can be found at <http://jobs.fzk.de> and at <http://ankaweb.fzk.de>

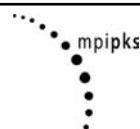
For more information, please contact Prof. Dr. Baumbach, T: +49 (0)7247 82-6820.

Contact: Kindly send your application, containing all documents relevant to this job (e.g. curriculum vitae, school/university certificates, training certificates, etc.), to Mrs. Hase, HPS, making explicit reference to the Vacancy No. 73/2006, 74/2006, 174/2006, 193/2006, 195/2006, 315/2006, or apply online at <http://jobs.fzk.de>.

Forschungszentrum Karlsruhe GmbH in der Helmholtz-Gemeinschaft Central Department for Personnel and Social Matters

**Prof. Dr. Baumbach or Mrs. Hase
T +49 (0)7247 82-6820,
T +49 (0)7247 82-5011
P.O. Box 36 40, 76021 Karlsruhe, Germany
Internet: www.fzk.de**

Max Planck Institute for the Physics of Complex Systems



In the department **FINITE SYSTEMS** of the institute

a long term position in Theoretical AMO Physics

is available. The successful candidate is expected to conduct independent research on the highest level and will also be responsible to coordinate the research area collision theory & cold atoms within the Finite Systems group (<http://www.pks.mpg.de/~rost>).

Postdoctoral experience, preferably abroad, is helpful but not mandatory. Habilitation at the TU Dresden is possible. The position is offered for 5 years with TVöD salary.

Please send your CV with publication list, a short description of research accomplishments and future research plans until **October 31, 2006** to:

Prof. Dr. Jan M. Rost

**Max Planck Institute for the Physics of Complex Systems
Nöthnitzer Straße 38
01187 Dresden, Germany**

Please arrange for two letters of recommendation to be sent directly to the address mentioned above.



MAX-PLANCK-GESELLSCHAFT

W89182R



Hannover Biomedical Research School

- leading research and teaching in Germany

**20 positions in the MD/PhD program "Molecular Medicine" and
20 positions in the PhD program "Infection Biology"**

Hannover Medical School (MHH), and the Helmholtz Centre for Infection Research, as part of Hannover Biomedical Research School, invite applications for the above PhD studentships, to commence in October 2007.

Both three-year study programs, taught in English, are aimed at postgraduates in Medicine as well as those from Life Science fields. Successful candidates will finish with a PhD, alternatively Dr. rer. nat. Scholarships are to be fully funded by the MHH, and partner institutes.

We are looking for highly-motivated candidates who have an active interest in one or more of the above fields. Excellent written and spoken English skills are required. With nearly two thirds of our students coming from outside Germany, international applicants are welcomed. Deadline for completed applications is **April 1st, 2007**. Please state upon application the program you wish to join.

Detailed info:

Daniel.marlies@mh-hannover.de; <http://www.mh-hannover.de/hbrs.html>

W87095R

North Rhine Westphalia Undergraduate Science Award

The NRW Graduate Schools have announced an undergraduate award to foster the early development of research experience and practice during undergraduate studies. Every NRW Graduate School awards an annual prize of 1,500 Euro for an outstanding research paper in the corresponding field of research.

Applicants should be co-authors of outstanding publications in high-ranking scientific journals. The date of acceptance of the paper should be in 2005 or 2006.

The students or faculty advisors to the respective NRW Graduate School from which the award may be obtained can submit applications for the award. For further information please visit:

<http://www.cebitec.uni-bielefeld.de/undergraduate-awards/>

The submission deadline is December 31, 2006.

The seven awardees (one for each participating graduate school) will be invited to receive the prize during an honorary reception at the North-Rhine Westphalian state capital of Düsseldorf.



W89309A

Max-Planck-Gesellschaft Max Planck Society



Selbstständige Nachwuchsgruppen

Independent Junior Research Groups

The Max Planck Society invites applications from outstanding young scientists in the field of **Biology and Medicine**

Successful applicants will have demonstrated the ability to perform excellent research. They will be offered an

Independent Junior Research Group Leader position

(W2; equivalent to associate professor level without tenure) including a five-year grant (research positions, budget, investments) at one of the following Max Planck Institutes

MPI for Medical Research, Heidelberg
(1 position)

MPI for Experimental Medicine, Göttingen
(1 position)

MPI for Biophysical Chemistry, Göttingen
(2 positions)

MPI for Developmental Biology, Tübingen
(1 position)

MPI of Immunobiology, Freiburg
(2 positions)

MPI for Molecular Plant Physiology, Golm/Potsdam
(2 positions)

MPI for Molecular Genetics, Berlin
(1 position)

Applications should include a CV, a list of publications, copies of three publications, a one-page summary of scientific achievements, and a two-page research plan.

For further information and detailed application instructions see

<http://www.snwg.mpg.de>

The Max Planck Society is committed to equal opportunities and to employing disabled persons.

The deadline for application is **October 31, 2006**.

W89141R



Miltenyi Biotec

Researchers working for researchers.

www.miltenyibiotec.com/jobs

Miltenyi Biotec, founded in 1989 near Cologne, Germany, has become a successful diversified biotechnology company. With subsidiaries and distributors around the world, the company has almost 1000 employees located in Germany, USA, UK, France, Benelux, Italy, Spain, Singapore, Japan, Australia, and China.

The product portfolio—more than 700 products—offers comprehensive solutions for basic, biomedical, and clinical research. Miltenyi Biotec develops, produces, and markets state-of-the-art reagents, instruments, and services for cell separation, cell analysis, cell culture, molecular biology, and clinical research applications.

The Miltenyi Biotec team members combine scientific expertise in cell biology, immunology, hematology, stem cell biology, molecular biology, bioinformatics, chemistry, physics, and engineering.

Miltenyi Biotec is constantly seeking top-quality personnel. The working environment is dynamic and challenging, with flat hierarchies and excellent career perspectives: www.miltenyibiotec.com/jobs.

Miltenyi Biotec GmbH

Friedrich-Ebert-Straße 68

51429 Bergisch Gladbach

Germany

www.miltenyibiotec.com

W89251R

GEORG SPEYER HAUS

Chemotherapeutisches Forschungsinstitut Georg-Speyer-Haus Frankfurt am Main www.georg-speyer-haus.de

The Georg-Speyer-Haus in Frankfurt am Main, Germany is an academic non-profit research institute supported by the Federal Ministry of Health and the Ministry for Sciences and Arts of the State of Hessen. The institute is dedicated to basic and translational research in the fields of cancer and infectious diseases, and has close ties to the University of Frankfurt and the Frankfurt University Hospital.

In the group of **Dr. Ursula Dietrich** and **PD Dr. Roland Stauber** three positions for **Doctoral Students (BAT IIa/2)** are available starting November 1st, 2006. The positions are funded by the European Commission within a "Specific Targeted Research or Innovation Project" (STREP) with the aim to exploit cellular export of nuclear transcripts as HIV innovative therapy. A strong background in Molecular Biology and/or Cell Biology is of advantage. **Contact: ursula.dietrich@em.uni-frankfurt.de**

In the group of **Prof. Dr. W. Wels** a position for a **Doctoral Student (BAT IIa/2)** is immediately available for a project concerned with the retargeting of immune effector cells to tumor-associated surface antigens via novel chimeric antigen receptors. For this position a Diploma degree in Biology or Biochemistry and experience in Molecular Biology are required. A strong background in Molecular Immunology and/or Cell Biology is of advantage. **Contact: wels@em.uni-frankfurt.de**

In the group of **PD Dr. Martin Zörnig** a position for a **Doctoral Student (BAT IIa/2)** is immediately available for a project concerned with the regulation of Fas Ligand function and the identification of novel anti-apoptotic proteins. A strong background in Molecular Immunology and/or Cell Biology is of advantage. **Contact: zoernig@em.uni-frankfurt.de**

Please direct applications including CV, university entrance qualification (Abiturzeugnis), university certificates, list of publications, brief summary of previous research experience and two letters of reference to:

**Chemotherapeutisches Forschungsinstitut
Georg-Speyer-Haus
Paul-Ehrlich-Straße 42-44
D-60596 Frankfurt am Main
Germany**

W89265R



Miltenyi Biotec

Miltenyi Biotec, founded in 1989, is an international biotechnology company based near Cologne, Germany, focusing on cellular and molecular biology technologies. With more than 900 employees worldwide, we develop, produce, and market state-of-the-art products and technologies for cell separation, cell biology, and related molecular biology applications.

Our extensive product portfolio includes products for research scientists and for clinicians, including reagents, instruments and medical devices.

In line with our constant expansion, and to strengthen the R&D team working to develop products for biomedical stem cell research and their clinical applications, we are seeking a:

Stem Cell Research & Development Project Manager (m/f)

Your responsibilities

As an R&D group leader, your main tasks will lie in the initiation, planning, organization, implementation, and quality control of projects with the aim of developing marketable products (reagents and consumables) for stem cell research as well as for the therapeutic application of stem cells.

Products include:

- Monoclonal antibodies
- Cell culture media
- Growth factors
- Cell separation reagents

Your Profile: You have completed a PhD in the field of stem cell research and have ideally 2–5 years of industrial or academic experience within the field of stem cell research and their therapeutic application (Postdoc, group leader). You possess an in-depth knowledge of the field and enjoy working within a team. You are a highly motivated character and can motivate others. You are creative and able to work in an independent, quality-oriented fashion, and are open to new challenges.

What we offer: As one of Germany's leading biotechnology companies that is constantly growing, we offer flat hierarchies, outstanding opportunities for personal and career development, and challenging duties with an opportunity to fashion your own responsibilities. In addition, you will be participating in the development of exciting new medical products for use within the field of cellular therapy.

If you are excited by what you have read and believe you match our criteria, please send us your full application including details of salary requirements and a possible start date to:

Miltenyi Biotec GmbH

Human Resources Department

www.miltenyibiotec.com

Friedrich-Ebert-Straße 68

51429 Bergisch Gladbach, Germany

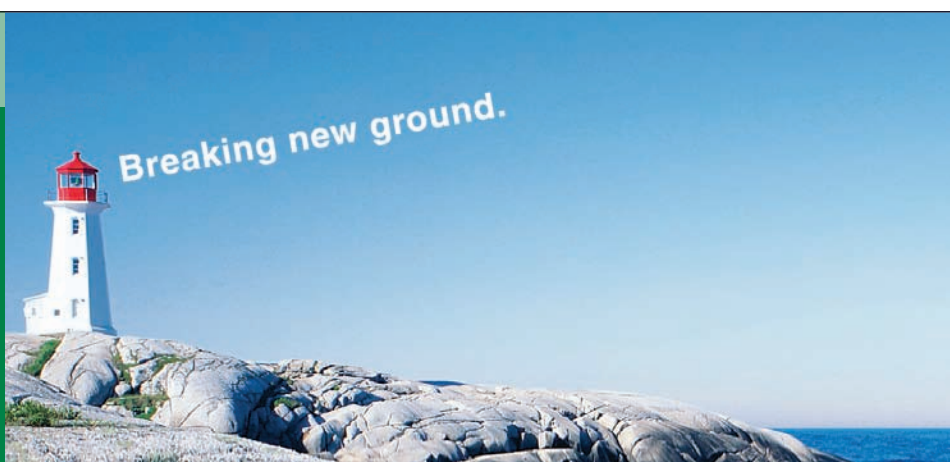
simoneg@miltenyibiotec.de

W88651R



Avoid getting
in it with
impressive
interview and
resume/CV advice

naturejobs



A person looking at things from different angles can create new ideas, new products, new technologies. As the world's leading chemical company, our products and innovations contribute to improved quality in various areas of daily life. Our success is based on qualified employees bringing their visions to life.

BASF Plant Science based in Ludwigshafen, Germany, is the plant biotechnology research platform of BASF and its partners. With over 600 employees worldwide in the increasing BASF Plant Science network, it is the aim of BASF Plant Science to become one of the internationally leading plant biotechnology companies.

BASF Plant Science GmbH is looking for dedicated employees, especially for the current vacancy:

Scientist Plant Biology (f/m)

Your responsibilities:

- Be an integral team member of interdisciplinary project teams working on bringing products to the market
- Planning research concepts and implementing them in the laboratory
- Heading a laboratory team

Your qualifications:

- Excellent knowledge in molecular plant biology, physiology and/or biochemistry
- PhD in biology/biochemistry/agronomics
- Excellent communication and teamwork skills
- Excellent English skills
- Working knowledge in German
- At least 2 years of postdoc experience

We offer you the active participation in developing our business in a very dynamic environment. Working in a motivated team with excellent career development opportunities within the BASF global network.

We look forward to your application!

If you have any questions please contact:

Ms. Michaela Hambsch
tel. +49 621 60-28102

Please apply preferably online under

www.basf.de/careers

or in writing to

BASF Aktiengesellschaft
HRdirekt
GPS/H - D 108
D-67056 Ludwigshafen
tel. +49 621 60-95200

Job-ID:
J-BPS-50692657

W89185R



PhD Studies at the International Helmholtz Research School of Biophysics and Soft Matter

Coordinators: U.B. Kaupp and G. Gompper

The International Helmholtz Research School on 'Biophysics and Soft Matter' (BioSoft) at the **Research Centre Jülich (FZJ)** in cooperation with the **Universities of Düsseldorf and Köln** will provide an in-depth training in Biophysics and Soft Matter and a comprehensive framework of experimental and theoretical tools that will enable Ph.D. students to gain deeper understanding into the structure, dynamics, and function of complex systems, in particular those related to living systems. The International Helmholtz Research School offers positions for graduate students (leading to a Ph.D.) for three years. Projects for a Ph.D. thesis can be selected from the following research areas:

- Complex Fluids
- Colloid and Polymer Physics
- Dynamics of Macromolecules
- Supramolecular and Amphiphilic Self-Assembly
- Flow Dynamics and Microfluidics
- Single-Molecule Detection and Manipulation
- Optical Spectroscopy and Imaging
- Bioelectronics and Biomechanics
- Cell Biophysics
- Structural Biology
- Cellular Signaling Pathways
- Receptor-Ligand Interactions
- Artificial and Biological Membranes

The Research School is open to highly qualified and motivated applicants from all countries.

Applicants should hold a Master's or other degree comparable to a German University Diploma in biological, chemical or physical sciences, and preferentially be under 27 years of age.

The teaching language is English; sufficient command of the language is required for acceptance.

Candidates will be invited for an interview with faculty members.

The Research School is committed to an equal opportunity policy. We therefore welcome and encourage the application of qualified female candidates. The school starts in the fall/winter 2006. Please forward your CV, including a score sheet (grades) and three names of individuals that can provide letters of recommendation to **Ms. Anja Mataruga: biosoft@fz-juelich.de**

For further information go to: www.ihrs-biosoft.de

W89173R



**Universitätsklinikum
Hamburg-Eppendorf**

Center for Molecular Neurobiology, Medical Faculty, University Medical Center Hamburg-Eppendorf invites applications for the position of a

Full Professor (W3) for 'Molecular Neuropathobiology'

(Ref. No. **FB04-62/3**)

The University of Hamburg is especially interested in the applications of women because it is intended to increase the proportion of female Professors. In line with the Higher Education Law of the State of Hamburg women will be given priority in case of equal qualification.

Description of the post

As the director of the Institute for Molecular Neuropathobiology at the Center for Molecular Neurobiology you are responsible for research and teaching. You are an internationally recognised expert in the field of Neuropathobiology. Areas of research using modern molecular biological approaches could include the following topics: neurogenesis; neuronal networks; pathogenesis of neurogenic tumours; molecular basis of hereditary diseases of the nervous system. It is expected that you will participate in teaching within the framework of an existing molecular biological graduate programme.

Conditions of Employment

In line with the regulations of the Higher Education Law of the State of Hamburg, the position is open to both German and foreign nationals. Evidence of postdoctoral lecture qualification or equal scientific credentials is required.

You have distinguished yourself as an outstanding and internationally recognised scientist in the field of Molecular Neuropathobiology. Apart from your academic profile you have high-level management and leadership skills. Special abilities and performances in teaching will be particularly considered. A description of your teaching experiences as well as a concept of teaching needs will be part of your application. A list of your current grant support should be added.

The initial duration of the term in office is six years; after an evaluation the contract can be extended to a permanent one. Remuneration is based on the German W3 salary for Professors.

Candidates with a disability will be given preferential treatment in case of equal qualification and academic performance.

Please send three copies of your application, including CV, references, short list of publications, description of teaching experience, concept of teaching, and list of current grant support to the Dean of the Medical Faculty of University Medical Center, Hamburg-Eppendorf, Gremienbetreuung - DG 1 -, Martinistr. 52, 20246 Hamburg, Germany.

Closing date: 10th November 2006.

W88750R

GEORG-AUGUST-UNIVERSITY GÖTTINGEN

Faculty of Medicine

UNIVERSITY CLINIC



The Faculty of Medicine and University Hospital of the Georg August University Göttingen is offering a

FULL PROFESSORSHIP IN NEUROIMMUNOLOGY (SALARY LEVEL W3)

at the Institute for Multiple Sclerosis Research.

The Institute is jointly funded by the Hertie Foundation and the Faculty of Medicine. The position is to be occupied immediately.

An outstanding scientist in the field of neuroimmunology is being sought for this position. In addition to carrying out his or her own research, for which a separate research group and laboratory space will be provided, the appointee is to coordinate research activities at the Institute of Multiple Sclerosis Research. Projects to be coordinated deal with the analysis of the molecular mechanisms of myelin and axonal damage in multiple sclerosis, the analysis of cytotoxic action between immune cells and neurons, and the development of new strategies for gene or cell-replacement therapy in animal models of multiple sclerosis. Close cooperation with other departments active in neuroimmunology research as well as with the Department of Neurogenetics and the Biomedical NMR-Research Group at the Max Planck Institute for Biophysical Chemistry is expected. The appointee should also participate in teaching at the Faculty of Medicine and University Hospital.

The University of Göttingen is sponsored by a foundation under public law. The requirements for appointment to professorship are documented in § 51 of the Higher Education Law of the State of Lower Saxony (NHG; Nds. GVBl. 1998, Pg. 318). Further details may be supplied on request. Habilitation or scientific achievement of an equivalent nature is necessary for this position.

On the grounds of a resolution passed by the Conference of the Ministry of Education and the Arts, the future appointment of professorial staff with medical responsibilities will be effected within the framework of non-union tariff-based contracts consisting of a basic salary as well as performance and success-related pay elements.

The University of Göttingen aims to increase the proportion of female scientific staff employed and thus invites applications from women in particular. In the case of similar qualifications, women will be considered on preferential terms within the framework of the legal possibilities. Preference will also be given to applications from disabled persons in the case of equal aptitude.

Applications, including curriculum vitae, an overview of your scientific career, teaching activities, a structured bibliography of publications relevant to this field together with offprints of the six most important articles, as well as evidence of successful external funding activities are requested within 3 weeks following the appearance of this advertisement to be sent to:

The Dean and Member of the Board of Directors for Research and Education of the Faculty of Medicine and University Hospital of the Georg-August-University Göttingen, Robert-Koch-Str. 42, 37075 Göttingen, Germany.

A short application form can be found under:
www.ukg-goettingen.de/content/804.html

W88756R

Want the best of the global market?

Make **naturejobs** your first choice.
making science work



SYSTEMS BIOLOGY

The Freiburg Initiative for Systems Biology FRISYS, a member of the FORSYS program of the Federal German Government, will be located at the new Centre for Systems Biology (Zentrum für Biosystemanalyse, ZBSA) in Freiburg, Germany.

FRISYS announces **three new Professorships** (Tenure track) available in January 2007 in the fields of:

- Theoretical Systems Biology and Mathematical Modelling
- Microbial/Prokaryote Systems Biology
- Biomedical Systems Biology

These appointments will come with a competitive package. For details on the forthcoming offering please visit our website at WWW.FRISYS.biologie.uni-freiburg.de after October 1, 2006.

We additionally encourage applications for the following open positions:

2 Lecturers (Tenure track) for the implementation of a MSc and PhD level educational program in Systems Biology.

After a 5 year period, appointees will be evaluated and may be offered permanent positions.

1 Head of Administration of the ZBSA (permanent position), with a scientific background and wide experience in university administration management. More information can be found at www.zbsa.de

1 Leader of the core facility Data Analysis and Modelling (5 years appointment)

Up to 20 post-docs and graduate students in the fields of Experimental and Theoretical Systems Biology (2-5 years appointments/fellowships).

We seek science graduates with a keen interest in both biology and mathematical modelling. Research focuses on Systems Biology experimental analysis and mathematical modelling of cellular signalling and decision-making systems. Applicants with expertise in Systems Biology, molecular biological, and/or computational biology will be considered.

1 Project Assistant (5 years). The trained scientist, preferably a biologist, will coordinate activities in the specific work packages of FRISYS, support the interaction of platform and modelling activities with specific projects. Furthermore, the report and administrative work, as well as the public presentation of FRISYS will be a major responsibility of this position.

Please visit our web site at WWW.FRISYS.biologie.uni-freiburg.de for job advertisements at Research technician and Technologist levels.

All academic positions are linked to one of the following faculties of the University of Freiburg: Biology, Mathematics and Physics, Computer Sciences and Applied Sciences, Medicine (groups of Hess, Timmer, Driever, Baumeister, Reski, Laux, Palme, Backofen, Reth, Benzing).

Assessments will start November 1, 2006, and continue until positions are filled. All positions are available starting January 1, 2007.

For further information and details on how to submit applications, please go to WWW.FRISYS.biologie.uni-freiburg.de

Contact information: sysbio@cyanolab.de

W89271R



Cenix BioScience is a leading biotechnology company specializing in advanced, genome-driven applications of RNA interference (RNAi). Spun-out in 1999 from top European research institutes (EMBL, Heidelberg, and the Max Planck Institute CBG, Dresden), Cenix has been fully self-sustaining since 2003, and profitable since 2004.

Our international team carries out cutting edge drug discovery research projects with leading industry and academic partners worldwide, from state-of-the-art facilities in the heart of Dresden's growing life science research community.

As the demand for our Premium Research Services is now exceeding our existing capacities, we are seeking to further strengthen our cross-disciplinary team by filling the following positions:

Associate Research Scientist for New Proteomics Profiling Unit (Pos. code PARS-C3PO)

You will:

- Be part of a new team developing methods and technologies to apply MS-based proteomic profiling analyses on RNAi- and drug-treated mammalian cell samples;
- Design, implement and analyse a wide range of cell-based experiments, reporting to the team's head, and assisting in build-up of joint facility with in-house academic partners.

But first, you need:

- Ph.D. or MSc with >2 yrs lab research experience including solid theoretical and working knowledge of mass spec-based protein analyses and/or associated biochemical separation techniques.

Associate Research Scientist for HT-RNAi Cell Screening Unit (Pos. code BARS-HTCS)

You will:

- Develop new methods for high throughput RNAi- and/or drug-based experimentation in cultured mammalian cells;
- Develop new HT cell-based assay techniques to monitor wide range of cellular processes, with emphasis on automated microscopy for all scales of experimentation.

But first, you need:

- Ph.D. or MSc with >2 yrs lab research experience in molecular cell biology;
- Solid theoretical and working knowledge of cell-based assays, immunofluorescence microscopy;
- Proficiency in advanced uses of Excel spreadsheets to manage and present large datasets;
- Strong initiative & organizational skills; (Individuals with experience in immunological or infectious diseases are encouraged to apply).

Technical Research Assistant for HT-RNAi Cell Screening Unit (Pos. code TRA-HTCS)

You will:

- Carry out wide range of microtiter plate-based experimentation, including plate reader- and automated microscopy-based assays and RT-PCR assays on cultured mammalian cells.

But first, you need:

- Technician or BSc training plus >2 yrs experience in mammalian cell-based research, including tissue culture and microscopy;
- Excellent concentration, strong attention to detail, good documentation skills;
- Strong ability to work in a team.

Software Developer for IT Unit (Pos. code SD-IT)

You will:

- Develop custom user interfaces for the Cenix Laboratory Information Management System (LIMS), a distributed, cross-platform application based on the wxPython GUI toolkit;
- Actively participate in all phases of the software development cycle in close coordination with a team of developers and lab scientists;
- Train and assist the scientists in using the Cenix LIMS and work on its integration with third-party software.

But first, you need:

- At least 2 yrs of object-oriented programming experience in a team;
- Advanced GUI programming skills, including a good understanding of user interface design principles, ideally in a biological research context;
- Expert knowledge of at least two of the three operating systems the Cenix LIMS is being used on (Windows, Mac OS-X, and Linux).

Cenix offers very competitive salaries and generous benefits. Our company working language is English, and therefore, all staff must be completely fluent. If you are interested in one of these positions, please email, send or fax a complete c.v. with 2 references or alternatively, referee contact details, quoting the position code to: Ellen Fuerst, Human Resources, Cenix BioScience GmbH, Tatzberg 47, 01307 Dresden, Germany. Tel: +49 (351) 4173 128, recruit@cenix-bioscience.com.

RNAi
www.cenix-bioscience.com



SFB/TR21

DFG

The Transregional Collaborative Research Centre SFB/TR21 "Control of quantum correlations in tailored matter: Common perspectives of mesoscopic systems and quantum gases" at the universities of Stuttgart, Ulm and Tübingen invite applications for an

Independent Junior Research Group Leader Position (salary scale BAT 1a)

The Research Group will be incorporated in the general research programme of the Research Centre SFB/TR21 (for further information see: <http://www.physik.uni-stuttgart.de/TR21/>), where the required laboratory/office space and basic equipment, including overheads, will be provided.

We intend to install an experimental group in the Project Area B: Controlling Quantum phase transitions and/or a theoretical group in the Project area C: Engineering hybrid quantum systems.

The candidate, a highly qualified junior scientist with a strong research interest in either project area shall hold a PhD, passed no longer than six years ago. We expect relevant research experience, an excellent scientific record, the ability to independently lead a research group, active collaboration and significant contributions to the research goals of the SFB/TR21.

The Independent Junior Research Group is funded by the Deutsche Forschungsgemeinschaft for up to five years and will enable independent research to be carried out within a research network. Funding includes the position of the group leader, scientific and technical personnel as well as consumables and instrumentation.

Evaluation includes a personal presentation of the project and panel assessment.

Applications should include a research plan (max. 5 pages: previous work in the field, goals, methods and research program, schedule, approximate cost break down), CV and a list of publications are to be submitted by **31.10.2006** to: Universität Stuttgart, SFB/TR21, 5. Physikalisches Institut, Pfaffenwaldring 57, D-70550 Stuttgart, Germany

For further details please contact: tr21@physik.uni-stuttgart.de

We wish to increase the proportion of female academic staff and therefore particularly encourage female scientists to apply. Handicapped persons with the same qualifications will be given preference.

W89170R

UNIVERSITÄT LEIPZIG

Medical Faculty

The **Interdisciplinary Centre for Clinical Research (IZKF)** at the University of Leipzig invites applications for the Position of

Independent Research Group Leader „Molecular Mechanisms of the Metabolic Syndrome“

to be funded up to BAT-O 1a level for a period of 5 years from 1st January 2007. Additional support is available for a post doctoral fellow (or two graduate students) and a technician, with an appropriate allowance for equipment and consumables.

The Centre is part of an interactive science network which includes the Biomedical-Biotechnological Centre (BBZ), the Coordination Centre for Clinical Studies (KKS) Leipzig, the Interdisciplinary Centre for Bioinformatics (IZBI), three Max-Planck-Institutes, the Environmental Research Centre Halle-Leipzig, the Fraunhofer Institute of Immunology and Cell Therapy (IZI) and the Bio-City Leipzig, which hosts life-science start-ups.

The IZKF conducts research into cell-cell and cell-matrix-interactions relevant to diagnostic and therapeutic applications, focussing on the specialist areas of immunology, endocrinology, neurosciences and oncology. The new group should establish a clear, cell-based approach to research in the field of molecular endocrinology, cardiology or angiology, and will be expected to take advantage of the excellent opportunities for collaboration within the IZKF, and with the Clinical Research Group (KFO 152) "Atherosclerosis".

In addition to a doctoral qualification in medicine or the life sciences, applicants must have a strong background in a relevant field of cellular endocrinology, experimental animal research, molecular cell biology, a clear interest in clinically orientated research, and the ability to attract external funding.

Applications are to include CV, publication list, a summary of planned research and 2 letters of reference (to follow if necessary).

Applications should be submitted within **3 weeks** after the appearance of this advertisement to:

**University of Leipzig
Medical Faculty
Bereich 4 Personal
Stephanstr. 9/C, D-04103 Leipzig**

Further details are available from the IZKF-Office, phone: +49-(0)341-97 15940, e-mail: izkf@uni-leipzig.de, www.izkf-leipzig.de

W89241R



Pioneering Science Delivers Vital Medicines™

Regensburg, Germany

Amgen, the world's largest biotechnology company, with over 16,000 employees worldwide, pioneered the development of novel products based on advances in recombinant DNA and molecular biology. Entrepreneurial and science-driven, we aim to attract and retain the most talented people who share our dedication to our mission of serving patients. At our research site in Regensburg, Germany, we focus on the discovery of small molecule drug candidates using high-throughput screening technologies and we now seek people who would thrive in an environment built on innovation, team work, risk-taking and the pursuit of excellence.

IT Support Analyst – Research Information Systems

A life sciences graduate, you should have three years' experience in a research laboratory as well as expertise in IT support.

Senior Scientists – Lead Discovery

We are looking for Biologists with at least three years' experience in the biotech or pharma industry as well as expertise in lead discovery.

Scientist – Lead Discovery

You should have a PhD in a biological discipline.

Senior Research Associate – Lead Discovery

You will need a diploma or masters in biology or biotechnology.

Please submit your electronic application (in English), including salary expectations and possible start date, to gwilde@amgen.com.

Amgen Research GmbH, Attn. Gabriele Wilde, Josef-Engert.Str. 11, 93053 Regensburg, Germany.

Phone: +49 (0) 941-465 20000

www.amgen.com

W89253R



LUDWIG-
MAXIMILIANS-
UNIVERSITÄT
MÜNCHEN

FACULTY OF GEOSCIENCES

The Ludwig-Maximilians-University Munich, Germany (LMU) invites applications for the position of

Chair (W3) in Paleontology and Historical Geology

The Chair is located in the Faculty of Geosciences and the Department of Earth and Environmental Sciences. This position is linked to the Directorship of the Bavarian State Collection of Paleontology and Geology and the Munich Paleontology Museum.

The successful candidate will contribute to teaching programs in the fields of paleontology and historical geology at graduate and undergraduate levels (B. Sc., M.Sc.), as well as post-graduate programs. It is anticipated that the successful candidate will be experienced in initiating interdisciplinary science, in academic administration, and in collections and museum work. Active participation in the GeoBio-Center^{LMU} is expected.

Applicants should be internationally renowned and have a strong scientific record in paleontology including quantitative methods in biostratigraphy, palaeoecology, geobiology, and/or biogeochemistry. Collaborations within the Munich GeoCenter (LMU-TUM) and other institutions should be fostered.

Applicants should hold a doctoral degree and have an outstanding teaching record as evidenced by a habilitation or be able to demonstrate equivalent qualification.

The candidate should be under 52 years of age at the time of appointment. Under special circumstances, exceptions are possible.

The LMU intends to increase the proportion of women in research and teaching, and hence strongly encourages female scientists to apply.

Priority will be given to physically disabled persons with equivalent qualifications.

If you have further questions or need additional information you may look to www.palmuc.de/ or www.iaag.geo.uni-muenchen.de/fak_website/fak/index.html or send an e-mail to Dekanat@geo.uni-muenchen.de.

Applications with CV, list of publications, university transcripts (including copies of diplomas and certificates), statement of teaching experience, reprints of three significant publications, and a description of research plans and philosophy should reach the **Dean of the Faculty of Geosciences, Ludwig-Maximilians-Universität München, Luisenstraße 37, 80333 München, Germany, by December, 31, 2006.**

W89037R

PhD Scholarships International Graduate School of Neuroscience (IGSN) Ruhr University Bochum GERMANY

Applications are invited for the IGSN PhD-Programme, which starts on October 1st 2007.

Research projects specialise in the areas of molecular, developmental, systems, cognitive, clinical or computational neuroscience, and comprise intensive training in state of the art methods, as well as supervision by internationally renowned neuroscientists. See www.rub.de/igsn for details.

Research projects will be accompanied by high-level english-language tuition in the field of neuroscience. The 36 month programme will culminate with a PhD in Neuroscience.

PhD-Scholarships (3 years, €15 330 p.a.) are available.

Application deadline: January 31st 2007.
Applications online via: www.rub.de/igsn

International Graduate School of Neuroscience
Ruhr University Bochum
Universitätsstr. 150,
D-44790 Bochum
Germany
igsn@rub.de



IGSN



W88432R



Center for Molecular Medicine University of Cologne

- from Basic Molecular Research
to Clinical Application -

The Center for Molecular Medicine at the University of Cologne (CMMC) offers a forum for competitive basic, disease-oriented research for scientists from the Faculty of Medicine and the Faculty of Mathematics and Natural Sciences.

The overall aim of our Center is to support state-of-the-art basic as well as translational research in molecular medicine in order to gain insight into pathogenetic mechanisms of diseases and to develop new and effective diagnostic as well as therapeutic approaches.

The main research areas are:

- A. Cardiac and Vascular Disorders
- B. Host Response to Tumor Growth, Inflammation and Infection
- C. Molecular Neurobiology

Training and support of junior scientists is a major commitment of the Center that involves a wide spectrum of strategies including the implementation of junior research groups and the establishment of an Interdisciplinary Postgraduate Program Molecular Medicine.

For further information please contact:

Dr. Debora Grosskopf-Kroiher (Scientific Coordinator)
Center for Molecular Medicine, University of Cologne
MTI-Hörsaalgebäude (44b), Joseph-Stelzmann-Str. 52
50931 Cologne, Germany, phone: + 49-221-478 6982
fax: + 49-221-478 4833, e-mail: cmmc-info@uni-koeln.de

www.zmmk.uni-koeln.de

W89262R



Federal Ministry
of Education
and Research

Junior Research Groups for Nutrition Research

The German Federal Ministry of Education and Research (BMBF) provides the opportunity to build up an independent research group in molecular nutrition for outstanding scientists, not older than 39 years, from Germany or abroad. The main objective is to contribute towards understanding the effect of individual foodstuffs and their constituents on human health. Functional Food of the future should add an individual benefit to human health.

Besides a strategically convincing scientific concept for new approaches to molecular nutrition research, applicants need a German research institution to host their independent research group. Officially the grant has to be awarded to a German institution. Depending on the proposed scientific concept the group may consist of 1 group leader, 1-2 postdoctoral scientists, 1-2 PhD students, technical assistants. Successful candidates will be grant-funded for 5 years. Proposals will be selected by a jury.

The closing date for applications is 30 November, 2006

Contact:

Dr. Henrike Boermans, e-mail: h.boermans@fz-juelich.de

www.fz-juelich.de/ptj/nachwuchswettbewerbernaehrung

W87727A

Leibniz Institute of Plant Biochemistry

Research on plant natural products



The large manifold of plant species is reflected in the enormous diversity of their natural products. This content of natural compounds is made more complex by the change in metabolite patterns during development as well as when a plant is responding to its environment. Knowledge of the structure and function of natural products is requisite to understanding plant diversity, developmental and adaptation processes. New resources can then become available for innovative application in plant production, plant protection, biotechnology, and in the development of biologically active compounds.

The Leibniz Institute of Plant Biochemistry is a publicly funded research institute located on the Weinberg Campus of the Martin Luther University Halle-Wittenberg, Halle, Germany. The institute provides

- essential infrastructure from the existing programs in stress and developmental biology (Dierk Scheel), natural product biotechnology (Claus Wasternack), bioorganic chemistry (Ludger Wessjohann), and secondary metabolism (Dieter Strack),
- state of the art technology for analytical, synthetic and structural chemistry, proteomics, metabolic profiling, biochemistry and molecular and cell biology,
- an energetic working environment with a multidisciplinary approach to natural product, molecular interaction, plant microbe interaction and gene function analyses.

The institute has openings for the following positions:

- Department Head
- Junior Research Group Leader
- Research Group Leader Metabolite Profiling

Please visit our homepage for more information:
<http://www.ipb-halle.de>

W89230R

INM Leibniz-Institut für Neue Materialien gGmbH

Das INM Leibniz-Institut für Neue Materialien gGmbH in Saarbrücken ist Mitglied der Wissenschaftsgemeinschaft Gottfried Wilhelm Leibniz (WGL) und ist eines der weltweit bekanntesten Forschungs- und Entwicklungszentren für neue Werkstoffe und chemische Nanotechnologien, dessen Spanne von der Grundlage bis zur industriellen Anwendung reicht. Es verfügt über eine hervorragende Ausstattung für Synthese, Charakterisierung, Analytik, Formgebungs- und Werkstoffprüfung und über Technikumsanlagen mit einer Gesamtfläche von 12.500 m². Für Ende 2006 / Anfang 2007 suchen wir mehrere

Doktoranden (m/w)

aus den Fachrichtungen Chemie, Chemieingenieurwesen, Materialwissenschaften und Polymer-Physik zur Bearbeitung von Themen aus folgenden Gebieten:

- Neue Polymer-Komposit-Werkstoffe und deren Anwendung
- Kolloide auf Basis von Keramik-Teilchen
- Folienoberflächen-Technologie
- Glas- und Keramik-Werkstoffe
- Sol/Gel- und Gasphasenmethoden zur Herstellung von Schichten

Die Vergütung richtet sich nach dem BAT bzw. TV-L. Wir fördern die beruflichen Chancen von Frauen und bitten besonders um ihre Bewerbungen.

Bitte senden Sie Ihre vollständigen Bewerbungsunterlagen an das INM Leibniz-Institut für Neue Materialien gGmbH, z. Hd. v. Herrn Bernd Rus, Im Stadtwald, Gebäude D2 2, 66123 Saarbrücken.

Weitere Informationen zum INM finden Sie unter www.inm-gmbh.de. Informationen zur Leibniz-Gemeinschaft finden Sie unter www.leibniz-gemeinschaft.de

W89260R

Do take the time to visit the Spotlights and Regions Page on **Naturejobs.com**

Here you can read through all the special features we have published until now.

<http://www.nature.com/naturejobs/regions/index.html>

Here is also where the current 'Highlight on Germany' will be visible.

CENTER FOR LIFE SCIENCE AUTOMATION

Innovative, interdisciplinary, international

Our profile

As an international center of expertise based in Rostock (Germany), celisca offers an ideal environment for effective interdisciplinary research and development projects. We concentrate, promote, and combine results and insights from various disciplines in order to increase knowledge and to develop better procedures and products for the life sciences more quickly than ever. Our work covers the entire range of life sciences, engineering and applications.

What we offer

- successful and innovative research in the automation of modern life sciences
- interdisciplinary projects in Automation + Engineering, Screening + High Performance Analytics, Chemistry + Biotechnology, Real Time Systems + Information Technology, Automation Assessment
- highly motivated and interdisciplinary team of specialists
- oriented to the needs of science and industry
- contract research and research collaborations

www.celisca.com



W88511R



**Ferdinand-Braun-Institut
für Höchstfrequenztechnik**

We research cutting-edge technologies in the fields of microwaves and optoelectronics. Our current research topics cover GaN and GaAs power transistors and MMICs for applications in mobile communications and space technology. The optoelectronic activities focus on high-power diode lasers for information technology, materials processing, as well as medical and space applications.

We offer positions for scientists /engineers

- GaN power-transistor technology & GaN MMICs
- high-power diode lasers

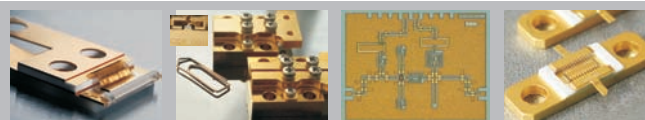
We are looking for highly qualified and strong team players with an university degree in electrical engineering, physics or a related subject along with appropriate professional experience. Further details are available on request: fbh@fbh-berlin.de

Please send your application, including curriculum vitae, list of publications and certificates to

**Ferdinand-Braun-Institut
für Höchstfrequenztechnik (FBH)**
Gustav-Kirchhoff-Str. 4
12489 Berlin, Germany

www.fbh-berlin.de

W89157R



MOVERS

**Haifan Lin, director, stem-cell programme,
Yale University, New Haven, Connecticut**



1994–2006: Assistant then full professor, Duke University, Durham, North Carolina
1990–94: Postdoc, Carnegie Institute of Washington, Washington DC

Haifan Lin made an early decision to pursue only projects that he felt passionate about. Physics and maths captivated him first. But when he heard the term 'genetic engineering', he thought he'd found the ultimate scientific feat.

While a biology undergraduate at Fudan University in Shanghai, Lin became one of the first Chinese students to win a joint China-US biochemistry fellowship. He went to Cornell University, where he was fortunate in his adviser, Mariana Wolfner. A new assistant professor, Wolfner was just starting her own lab, focused on gender-specific gene expression. But she supported Lin in studying the genes responsible for initiating embryonic cell division. His success vindicated the risks he'd taken. "It also gave me the confidence that I could get a new project to work," he says.

Seeking a fresh way to study cell division in development, Lin chose to work on stem cells as a postdoc with Allan Spradling at the Carnegie Institution of Washington, years before these cells became a household term. Although most such work was being conducted on mammalian cells, Lin focused on identifying stem cells in the fruitfly. He found that stem cells were not autonomous, as had been thought, but could have their function controlled by adjacent cells.

His achievements in stem-cell research paved the way to a faculty position at Duke University in Durham, North Carolina. There, Lin respectfully disregarded warnings from colleagues not to spread himself too thin, and opted to extend his stem-cell research in mammalian and even clinical directions. "I wanted to pursue interests that allowed me to see the bigger picture," he says. But, he adds, it was more difficult than starting a new lab in his field of expertise. "We had to start over from square one — even learning how to determine the gender of mice," he says.

In 1999, Lin started working with Duke researchers and clinicians to create an informal stem-cell programme, which eventually led to a university-supported programme co-directed with Brigid Hogan. He recently left to head a similar programme at Yale University. After a year of strategic planning, Yale leaders and Lin have created a new centre focused on fundamental stem-cell biology.

Lin's courageous approach to taking on new areas of research set him apart from other candidates, says deputy dean for scientific affairs at Yale's School of Medicine, Carolyn Slayman. But, his optimism, curiosity and, most importantly, his energy, she says, convinced everyone that he would set Yale's work apart from others.

Virginia Gewin

RECRUITERS & ACADEMIA

Investing in people

As the biomedical research enterprise evolves, funders' portfolios must evolve along with it. We need to respond to the growing importance of multidisciplinary research, consider how to nurture research in translational medicine, and make sure gifted young researchers are given sufficient opportunity to establish their careers.

The Wellcome Trust traditionally gives high priority to supporting individual scientists, through training programmes and career fellowships, which currently account for more than 20% of its funding. But to ensure that our funding schemes reflect emerging trends in biomedical science, we recently revamped our personal-support funding portfolio.

Establishing a research group is an important step. The Wellcome Trust's research career development fellowships, like most such awards in Britain, are for people who have held at least one postdoctoral position. But some high-flyers are ready for independence earlier. Our new Sir Henry Wellcome fellowships are for people within the first year of getting their PhD. These four-year awards will give postdocs ample freedom to tackle important biomedical questions in leading labs in Britain or overseas.

Young clinicians wishing to start a

research career in Britain can apply for a Wellcome Trust research training fellowship, but the quality of training environments does not always match the calibre of candidates. Meanwhile, research training for medics is at a premium. With clinical training also undergoing a major shake up in Britain, there is a need for a strong, integrated approach to clinical research training. We are launching a competition to host PhD programmes for clinicians, based in labs where research and training is of proven excellence. These will complement existing fellowships and help medics to identify first-class supervisors and mentors.

Opportunities for researchers to collaborate, learn skills and obtain experience in other disciplines are also crucial, particularly with the growth of inter- and multidisciplinary research. Our new flexible travel awards will help established scientists take sabbaticals and provide travelling fellowships to those without established posts.

Biomedical research has become a competitive, global endeavour. We hope our revised portfolio better reflects the challenges and opportunities for research in the twenty-first century.

Sohaila Rastan is director of science funding at the Wellcome Trust.

GRADUATE JOURNAL

Sounds of scientists

Heading downstairs late one night to concentrate DNA in the speed vac, I thought I had finally lost it after too many long nights in the lab. As I was putting my samples in the machine, I thought I heard someone bellowing show tunes from around the corner. Poking my head into the adjacent lab space, I discovered a now mortified friend of mine, Sandra, singing along at full tilt to the soundtrack of the musical *Wicked*. That's when I realized that maybe she had lost it.

Alternatively, perhaps we're both still at least somewhat sane. The truth is, I too have been known to carry a tune or two. Usually I sing along with the big speakers in the lab blaring, when I think no one is around. Occasionally I slip up when I have headphones on and everyone is around. It's never musicals, though — folk rock is more my thing. The exact choice of tunes changes with my mood. I spent one rather bleak month of unsuccessful experiments soulfully intoning Bob Dylan's *Highway 61 Revisited*. By contrast, after a particularly long and fruitful day of experimenting you'll find me rocking out to Bruce Springsteen's *Better Days*.

I suspect Sandra and I aren't the only lab crooners. We all have to vent our emotions somehow. I've spent a lot of hours talking to my yeast, some in joy and many in frustration. At least Springsteen knows how I feel. Refer to his recent release: it's titled *How can I keep from singing?*

Milan de Vries is a molecular-biology graduate student at the Massachusetts Institute of Technology.

Twenty2

The city without secrets.

Nate Balding

"Berlin is the new American experiment."

Vocal intonations; voluminous, deep, resonating inside the monorail car, translations from German into a multitude of languages tickling foreign cochleas lightly from translucent earbud speakers. A short history of Berlin since the erection of the Mitte Arcology and the subsequent construction of similar towers, falling away from the central point like evergreens on a mountainside; reinforced faux-glass impervious to high winds, acid rains, falling space-rock and high-frequency waves topping every pellucid spire.

Sprita's fingers circled a hollow titanium alloy bar running the length of the magnetic train's passenger chamber, body poised to offset any recoil.

"Our citizens, ten years ago, voluntarily became subjects of the Panmetro-polis Drive. There are no more secrets in Berlin."

Sprita stared through the monorail's tinted windows, watching information scroll over plexiglass as the train passed businesses and points of interest, relaying site info just that side of too-fast-to-read. She assumed it was easier for citizens, with their technological accoutrements gifting enough additional processing capability to endure the informational onslaught constantly pressing into the visual and aural cortices. It induced a headache the first day she'd been there, taking a holiday on the European continent with thousands of others who wanted to see firsthand the world's foremost social experiment.

"The Wall was built during the construction of the first arcology and lined with plates of copper and gold. Inside it, thousands of ferrous bullets fly alongside the edges, generating a massive electromagnetic field that engulfs the entire city. Every person living inside it is coded to communicate with what is essentially a city-sized geomagnetic hard drive."

The monologue continued. Sprita gazed at the other riders, easily discerning Berliner from visitor. Pronounced electronic excesses aside, the Berliners bore certain uncanny homogeneities. Similar hairstyles; similar dress; similar everything. Even the way they sat, practically posed: feet forward, parallel legs, hands in laps occasionally shifting intertwined over chests, and then back.

She'd picked up an oLED-equipped teleshirt. Everything you needed to know could

be played into an inlay along the sleeves, RSS'd in Arial MT. A miniature RFID tag issued on arrival stored the mainstay of her characteristics, physical and political, and launched her into the hard-drive world amid every other inhabitant, long- and short-term alike. Grant of the pass made her a subject to the experiment and she could, as everyone else, ping information back to a viewing screen or through the earbud she wore. Everything one wanted to know was easily available, constant wi-fi interaction conveyed everything to anyone upon query. Each and every purchase, location, trajectory, ping, glance; recorded, available. The city without secrets.

"Berlin is the only city in the world that can truly call itself twenty-first century. We are at the apex of a technologically advanced civilization, surpassing all others in scientific research and discovery. An entire city of would-be scientists and engineers, our collective knowledge available to anyone willing to share their own."

She was heading into the central arcology seeking a club called Twenty2. Her *personenkult* card informed her, based on commercial purchases and local browsing data, that Panda Parade was playing that night — one of Berlin's only surviving neo-Höllenspektakel groups. They'd exposed the viscera of their guitar pickups to heavy subdermal magnets — a common remanufacturing of the human hand — manipulating strings at varying frequencies, phalangeal theremins operating at resonant frequency and scrying ambient tones from an otherwise maniacal cacophony. The phenomenon started in the heart of Berlin and spread outwards, enthralling the youth of western Europe and slowly trickling on to sonic radar screens in America.

The monorail approached Sprita's stop. Twenty2 lay on the outskirts of the central arcology, in the detritus of last century's dimly illuminated gothic bars, primitive rave clubs and sparkling house-music meccas. Sprita drifted over the threshold as the magdoor bisected and swivelled aside, bodies pressed coarsely together, flash-throng shopping evidently under way as markdowns and sales and other notifications of imminent mercantile importance

scattered into the district, setting off mp6 alerts and charging buyers fractions of cents for personalized content. Her low-heels clicked and flickered over ceramic tiles comprised of super-atomic structures manufactured via bacterial scoring and forced bonds; elements not found in the leylines of the old periodic table.

Signals bounced off Sprita, tiny inversions of field straining against the vibration feature of her teleshirt tingling nerve clusters around her wrists, minuscule exosense shivers. She saw him from across the floor of the dark stage, multicoloured lights ambushing retinas at calculatedly odd moments, glaring and burning away. Their loop was positive, feeding back on each other in the holographic nightstream of virtual knowledge. Relationship status; exact weight and height; recent purchases what and where; living space; names of friends and degrees of separation between him and

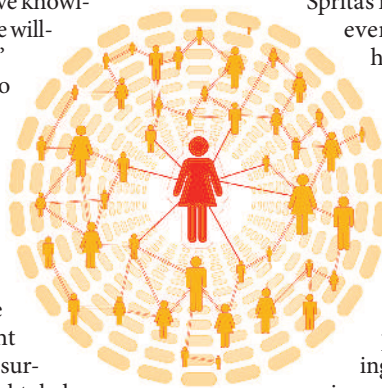
Sprita's nearly vacant friends list; everything grinned across her sleeve between ulna and radius.

He dug the music, she knew. So did she. It was almost dancing; faint glances parried by strobelights, vForm 2.0 imagery, carefully constructed coifs. Another boy's ping; another; reciprocating and reading and analysing, unearthing familiarities.

Recollections sparked and breathed; latent epiphanies in the corridors of her neural puzzle. So many; so many familiar likes and dislikes, favourite movies and books and bands and supermodels; political affiliations and angles of personal pictures. So much genetic differentiation between them all. So little cultural.

Acute paranoia developed and Sprita edged backward, vying for the club's front exit. She stumbled into the enclosed, recycled air of 'outside' and breathed deeply, gulping, nearly panicked. "I'm so foreign," she thought. "So, so foreign." Her eyes reflected the club's glowing versicolour logo while Sprita wondered if her hotel room had locks.

Nate Balding writes a zine called *Nico on Sunday Mornings*, knows how to rock, stays up later than you and drinks scotch instead of anything else. Reach him at NicoOnSundayMornings@gmail.com for enquiries.



JACEY

Abstractions



FIRST AUTHOR

The forests in the Guiana Shield and the Amazon basin of northern South America are probably Earth's largest stores of biodiversity. To explain large-scale

biogeographic patterns and processes, Hans ter Steege of the Institute of Environmental Biology in Utrecht, the Netherlands, and his collaborators looked at seven forest inventories of biodiversity compiled by countries in the region. They analysed the adaptation of the most common tree genera to soil fertility and dry-season length (see page 444). Here, ter Steege tells *Nature* about analysing the regions' rich biodiversity.

Why were the inventories first made?

To evaluate national resources such as timber. The Brazilian inventory is the biggest. It is used, for example, for agricultural purposes to estimate the quality of a soil and its mineral content for mining.

Why is it important to link gradients in factors such as soil fertility to tree distribution?

Linking two gradients helps us find the factors and mechanisms that influence diversity and species distribution. This is the first time such an analysis has been done on a large scale.

Do the participating countries have an interest in biodiversity?

Yes. Most have established a system of natural protected areas, especially Brazil and Venezuela. In Brazil there is also much interest in evaluating forest resources such as rubber. In other countries, such as Venezuela and Suriname, the main focus is conservation.

Was it difficult to collaborate with so many different nationalities?

No. We have been working with the Amazon Tree Diversity Network for a number of years. This consists of 50–60 people. The rules are clear — anyone contributing data becomes a co-author on resultant publications.

In future, do you plan to go into finer detail?

Yes, but the large-scale inventories are only detailed enough to look at the genus level. We are using one-hectare plots to look at the species level. We want to gather information about tree diversity that can help us understand primate nutrition. If we can calculate the number of trees that fix nitrogen, we can estimate the number carrying fleshy fruits that primates eat. We can then compare this with primate diversity and density.

Are we facing a biodiversity crisis?

I believe we are. I think the fragmentation of the Amazon is a problem and a pity. But the survival of the people living there must also be considered. ■

MAKING THE PAPER

Benoit Deveaud-Pledran

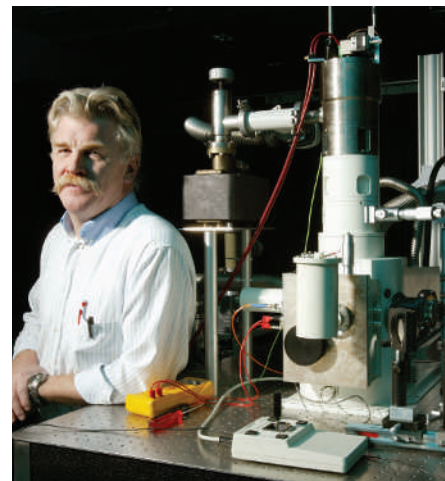
Physicists show possibility of creating superatoms from solids.

A Bose–Einstein condensate is matter in a phase distinct from solids, liquids and gases — it can be brought about from some gases at very low temperatures. When sufficiently chilled, most atoms in such gases drop to the same quantum level and essentially act as one overlapping atom — a 'superatom'. Although scientists achieved such condensation in rubidium gas more than a decade ago, efforts to slow particles in solids in a similar manner have been unsuccessful. This is because the temperatures solids can reach are constrained by limits to their disorder.

Now, collaborators from five institutions have come tantalizingly close to achieving Bose–Einstein condensation in solids. A group of physicists, led by Le Si Dang at the University of Joseph Fourier in Grenoble, France, used different particles from those conventionally used in such attempts. They made a subatomic trade-off. Excitons — neutral atoms in an excited state — theoretically require prohibitively low temperatures to condense, but are relatively stable. Short-lived particles known as exciton polaritons, which result from the coupling of light with excitons, do not require such low temperatures. But the condensate they achieve has a lifespan of picoseconds.

The group's results (see page 409) do not show a strict Bose–Einstein condensate. However, they provide evidence that such a state can be achieved in solids — albeit by different rules from those that apply to gases. When the researchers increased the polariton density, they saw signs of a transition toward a condensate. This condensate was coherent through space, albeit for a short time.

Benoit Deveaud-Pledran, a physicist at the Ecole Polytechnique Fédérale in Lausanne,



Switzerland, expects this work to be challenged. Previous claims by researchers that they had achieved solid-state Bose–Einstein condensation underwent vigorous attack. He predicts critics will contend that the particles he and his colleagues used have too short a lifespan to reach a stable condensate. "A number of people told us this was not possible," he says.

To bolster their claims, the group plans to make its data available to theorists outside the collaboration. These detail the fraction of polaritons that condensed at various temperatures. But the team has already suffered a setback — the machine used to produce its samples no longer works. The researchers hope the publication of this paper will attract funds for another machine. Until then, they hope that the evidence put forward in the paper will be enough to convince the sceptics.

Deveaud-Pledran is certain that Bose–Einstein condensation will continue to captivate researchers. He says that the past decade of dedicated basic-research funding has been vital to his work, which has also benefited greatly from recent theoretical advances. "Our long-term collaboration focused on the experimental side, but when things started getting hot, we contacted our theoretical colleagues to ask for their interpretation," says Deveaud-Pledran. ■

KEY CONTRIBUTOR

After finishing high school, Alina Vrabioiu travelled from Bucharest in Romania to the United States. There, she studied at the Massachusetts Institute of Technology, and quickly found success.

Just months after receiving a PhD in biology from Harvard, Vrabioiu, now a research fellow at Harvard Medical School, co-authored a *Nature* paper with her advisor, Tim Mitchison. He is well known for his work on cellular cytoskeletal dynamics.

Her project went surprisingly well, she says, allowing for a rare two-author paper in a field that often requires large teams of researchers. "The project was risky, but we were lucky," Vrabioiu says. "It's rare to just think of a project and then find it works."

Vrabioiu's paper describes the organization of filamentous septin proteins at the start of cytokinesis, the final stage of cell division in most eukaryotes (see page 466). The topic

has long been controversial. By rigidly attaching a green-fluorescent-protein marker to yeast septins, then probing them with polarized light, Vrabioiu and Mitchison were able to look at the septin filaments in fine detail.

They found that the filaments form ordered structures and undergo a 90° rotation just before the onset of cytokinesis. Their results indicate that these proteins have a mechanical role in cell division. ■